1995

Synthesis and Characterization of Water Soluble Chitosan Derivatives.

Javier Macossay
Louisiana State University and Agricultural & Mechanical College

SYNTHESIS AND CHARACTERIZATION OF WATER SOLUBLE CHITOSAN DERIVATIVES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemistry

by
Javier Macossay
B.S., Universidad Autonoma de Nuevo Leon, 1989
M.S., Louisiana State University, 1993
December 1995

This Dissertation is dedicated to my wife, Alma Delia, who has been supportive throughout the quest of achieving this dream, and has given me two great babies. To my children, Alma Maria and Javier Jesus, who have taught me that there is something more important than Chemistry in this life, and have given me the opportunity to learn new things and enjoy life. To the memory of my father, Javier Macossay Negrin, I wish you were here, miss you so much. To my mother in law, Maria del Roble, who has always encouraged me in more than one way to pursue my goals, and has cheered me up whenever I am down. And last but not least, to my mother, Yolanda, who made enormous sacrifices to give me an education.

THANK YOU

I LOVE YOU

ACKNOWLEDGMENTS

This Dissertation could not have been possible without the guidance of Dr. William H. Daly. I would like to thank Dr. Ioan I. Negulescu for his friendship and assistance throughout my graduate studies. Dr. Harold I. Weingarten for his friendship and schooling in teaching. Dr. Paul S. Russo, Dr. Roger A. Laine, Dr. John R. Collier and Dr. Samuel P. Meyers for being my committee members.

I am indebted to Javier Nakamatsu Kuniyoshi, Frederick H. Roos and Xiaolan Wang for sharing their friendship with me. I am grateful to Dr. Doris Culberson with whom I shared the frustration of working with cellulosics, and Mr. Marcus Nauman whose patience is greatly appreciated in teaching and assisting me with the NMR determinations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF PLOTS

ABSTRACT

Chitin, one of the most ubiquitous natural polymers, remains underutilized. The integral part chitin plays in natural biocomposites suggests that synthetic composites incorporating chitin should exhibit interesting properties, both with respect to strength and biodegradability. Due to a very strong hydrogen bonding, chitin is a very intractable material, but its derivative, chitosan, is very amenable to chemical modifications. Controlled deacetylation of chitin produces water soluble chitosan that is very efficient in complexing metals and natural biomolecules; major applications in water purification are reviewed.

In an effort to produce more water soluble derivatives of chitosan, with potential applications in the cosmetics and flocculation industries, we have evaluated the reaction of alkyl oxiranes with chitosan. We have prepared under basic and heterogeneous conditions hydroxyethyl chitosan, hydroxypropyl chitosan, and 2-hydroxypropyl trimethylammonium chitosan chloride, which are water soluble. More lipophilic derivatives such as hydroxybutyl chitosan and hydroxy(2-phenyl)-ethyl chitosan could be produced under acidic catalysis; however these derivatives are only soluble in acidic media. Synthesis of

cyanoethyl chitosan and its subsequent derivatization to aminopropyl, carboxyamidoethyl and carboxyethyl chitosan was achieved, but their solubilities in water and polar organic solvents are more limited than the oxirane derivatives. A method to promote decrystallization of chitosan, which increases its reactivity, prior to its derivatization is also presented.

CHAPTER ONE

INTRODUCTION

Natural macromolecules containing carbohydrate units perform a variety of tasks in living organisms, some of them yet to be understood. These macromolecules include: a) polysaccharides (as exclusively carbohydrate polymers); b) glycoproteins, proteoglycans and peptidoglycans; c) glycolipids and lipopolysaccharides; d) teichoic acids and e) nucleic acids.[7] However, not all these molecules are considered polysaccharides. The term "polysaccharide" is applied to those carbohydrate polymers that contain periodically repeating structures in which the dominant, but not necessarily exclusive, interunit linkages are of the O-glycosidic type. In this context, the term "polysaccharide includes: a) polysaccharides (as exclusively carbohydrate polymers); b) proteoglycans; c) peptidoglycans; d) lipopolysaccharides and e) teichoic acids.[7]

Polysaccharides are natural substances produced by microorganisms (dextran or xanthan), by primitive and higher plants (alginic acids, carrageenans, guar gums, cellulose, starch) and by mammals and crustaceans (heparin, glycogen, chitin). These

biopolymers serve living organisms in three different ways: as storage materials, structural components, and protective substances. Starch and glycogen, found in plants and animals respectively, are well known food reserves which may metabolize rapidly when needed.[8] Macromolecules such as cellulose and chitin play an important role as structural molecules and as links between other cell wall components. Polysaccharides used as protective substances are exemplified by the antigenic and immunogenic extracellular polysaccharides from microorganisms, and by the gums secreted by plants when sealing off injured parts from microbial infection.[7]

Polysaccharides, which constitute one of the most abundant and diverse families of biopolymers, may be viewed as condensation polymers where monosaccharides are joined together by glycosidic linkages with the elimination of water:

$$n\ C_6H_{12}O_6 \rightarrow (C_6H_{10}O_5)_n + (n\text{-}1)\ H_2O$$

Polysaccharides formed from a single monosaccharide unit are called homopolysaccharides or homoglycans, i.e., glucan (from glucose), arabinan (from arabinose) and xylan (from xylose). Polysaccharides containing more than one type of monosaccharide unit are called

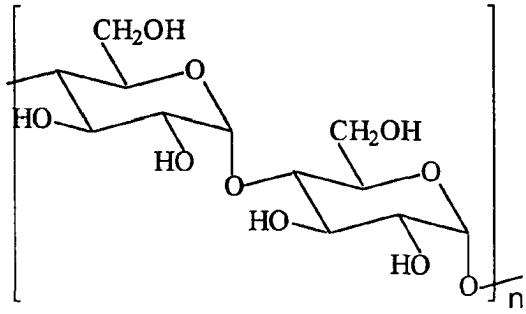
heteropolysaccharides or heteroglycans, i.e., arabinoxylans (from arabinose and xylose) and galactomannans (from galactose and mannose).

1.1 Plant polysaccharides.

Starch is a food reserve macromolecule which has been found in some protozoa, bacteria and algae. However, the major commercial sources of this biopolymer are plants, where it occurs as microscopic granules in roots, tubers, fruits, leaves and seeds. It is isolated industrially from sources such as corn, potatoes, wheat and rice, by heating its granules with water, causing them to swell and eventually to burst forming a paste.[8,10] The starch granule consists mainly of two polysaccharides, amylose and amylopectin, both containing (1→4)-α-D-glucopyranosyl residues, with the majority of the starches yielding between 15% and 35% of amylose.[10]

Amylose contains a mostly linear (1→4)-α-D-glucan found in various crystalline forms (Scheme 1.1).[9] It has several properties which can be explained in terms of the ability of this polysaccharide to adopt different molecular conformations in solution. In neutral aqueous solutions the normal conformation is random coil, but in the presence of

complexing agents in solution, amylose will form a helical structure consisting of about six D-glucosyl residues per helical turn.[10] This conformation complexes with fats and polar organic solvents, and is responsible for the blue coloration of amylose-iodine complexes.

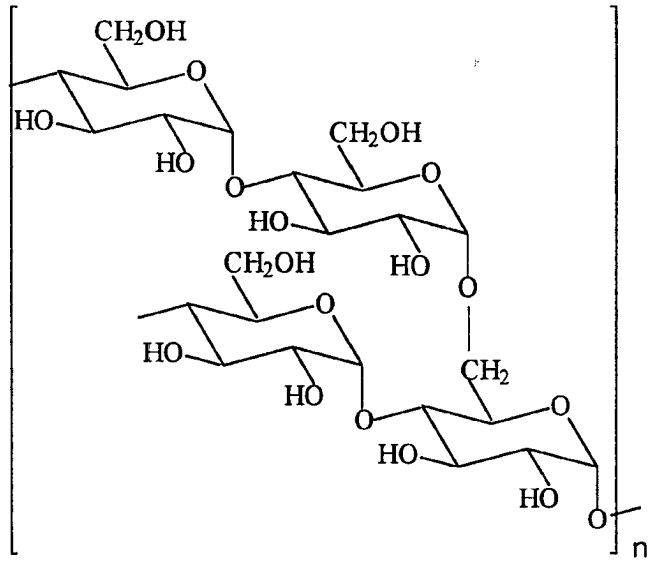


Scheme 1.1 Amylose

Unlike amylose, amylopectin is a branched, polydisperse glucopyranosyl polymer (Scheme 1.2). It contains an amylose-type (1→4)-α-backbone with clusters of (1→6)-α-glucopyranosyl branches with an average length of 20-30 residues.[9] This branched structure increases the density of end glucopyranose units available as energy sources, which can be released rapidly by exocyclic enzymes.

Cellulose, an important carbohydrate because of its high structural strength, is the most abundant polysaccharide found in nature (Scheme 1.3). It is the principal constituent of cell walls in higher plants, providing thus the primary framework of most of them. It occurs in

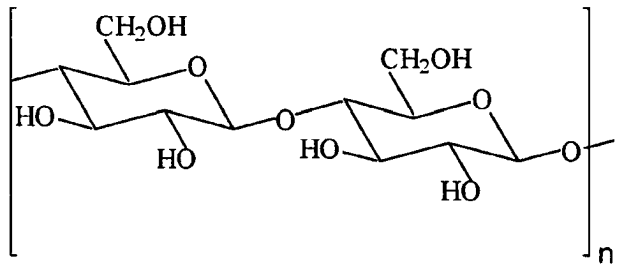
almost pure form in cotton fibers, and to a lesser extent in flax, jute and wood.[10]



Scheme 1.2 Amylopectin

The (1→4)-β-D-glucopyranose units in cellulose form a long unbranched polymer, which is essentially linear and does not tend to coil into helical structures as glucose polymers do when linked in a (1→4)-α manner.[8]The molecular chain length is high, and the intra- and inter-molecular hydrogen bonding capacity of the hydroxyl groups is optimized when two or more cellulose chains make contact, giving a highly insoluble, rigid and fibrous polymer.[10]

For industrial purposes, cellulose is isolated from cotton linters and wood pulp.[11] Cellulose derivatives are used commercially in different applications, for example, cellulose acetate is the cellulose ester with the largest commercial consumption worldwide. It is widely used in plastics, sheeting, films, cigarettes, photographic films, lacquers and the textile industry. The acetate-propionate and acetate-butyrate mixed esters have commercial applications as well.[11,12] Cellulose nitrate, the oldest and most important inorganic ester of cellulose, is used in the explosives, plastics, lacquers, and adhesives markets depending on the degree of substitution.[11,12] When regenerated cellulose is the objective, cellulose is reacted with carbon disulfide under basic conditions. The cellulose xanthate intermediate is then extruded into an acid bath, where the hydroxyl groups are regenerated, producing a fiber (rayon) or a sheet (cellophane).[8]

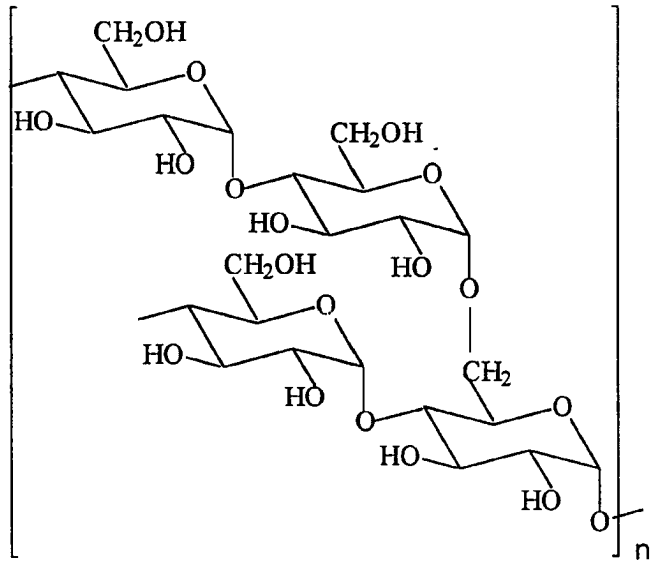


Scheme 1.3 Cellulose

1.2 Animal polysaccharides.

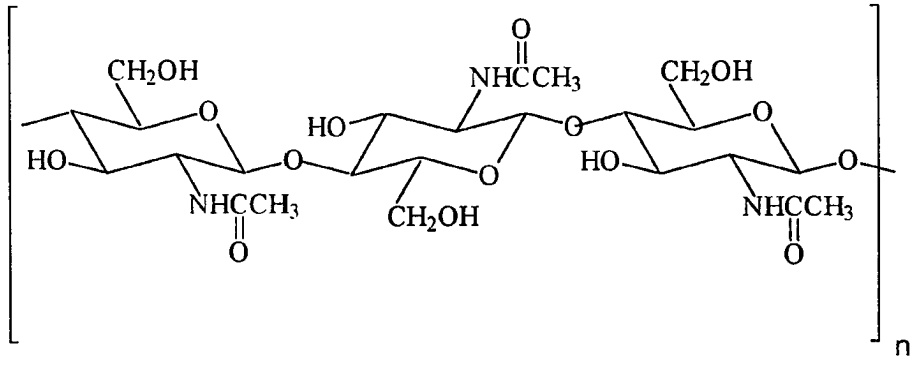
Glycogen is the principal food reserve polysaccharide found in animals (Scheme 1.4). Its (1→4)-α-D-glucopyranosyl chains and (1→6)-α-linked branches resemble those of amylopectin, but glycogen contains fewer branches and shorter chain lengths,[9] which result in markedly different physical properties. Further, the isolation and purification of glycogen are more complex than for starch. Glycogen is a highly water soluble, high molecular weight polymer that is mainly stored in the liver and skeletal muscle.[13] Its synthesis and degradation processes are important because they regulate the blood glucose level and provide a reservoir of glucose for strenuous muscular activity.[13]

Chitin, the most abundant polysaccharide containing amino groups, is the second most abundant polysaccharide found in nature (Scheme 1.5). It occurs as a structural polysaccharide in the shells of insects, crustaceans, mushrooms and yeasts.[14] Chitin is a homopolymer analogue to cellulose, with (1→4)-β-2-acetamido-2-deoxy-D-glucopyranose linear chains that adopt highly ordered chain conformations.



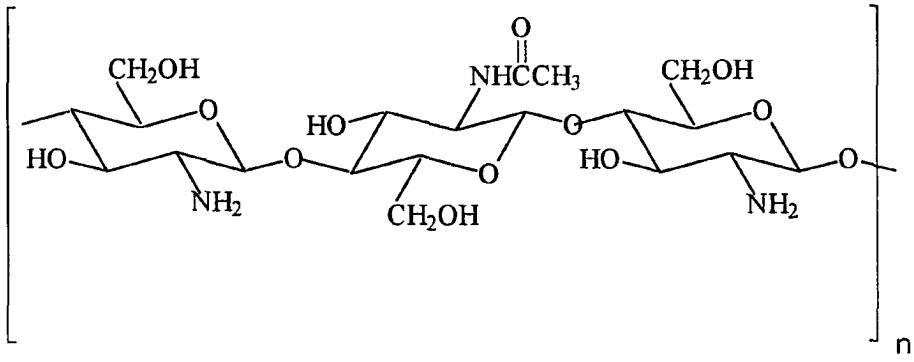
Scheme 1.4 Glycogen

The acetamido groups in chitin form strong intra- and inter-molecular hydrogen bonding with the adjacent hydroxyl groups, which accounts for the fact that chitin is insoluble in water and most organic solvents, thus hampering its derivatization and commercial applications



Scheme 1.5 Chitin

Chitosan, (1→4)-β-2-amino-2-deoxy-D-glucopyranose, is the partly deacetylated chitin derivative, which is obtained from an acid treatment followed by a basic amidolysis (Scheme 1.6). Although this biopolymer contains intra- and inter-molecular hydrogen bonds, they occur to a lesser extent than in chitin, affording a more soluble and reactive polysaccharide.



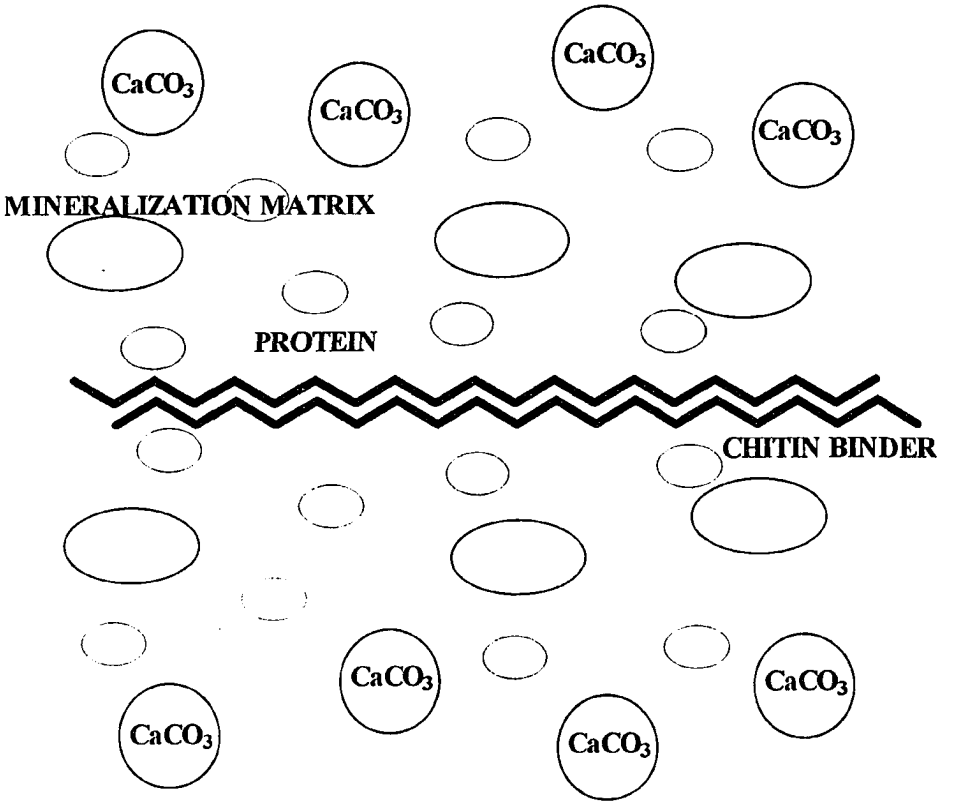
Scheme 1.6 Chitosan

The difference between these polymers lies in the percentage of acetamido groups at carbon 2, which varies with the conditions used to produce chitosan. While chitin has almost exclusively acetamido groups, chitosan is a copolymer containing acetamido and primary amino groups, which can be protonated to produce soluble polyammonium salts. Due to early confusion in the nomenclature of chitin/chitosan, most publications

use the term chitosan, as an attempt to be consistent, when the degree of deacetylation is more than 70%.[14]

1.3 Isolation of chitosan.

The vast amount of crustacean shells (crab, shrimp, krill and crawfish) available as a byproduct of the seafood industry, and the fact that the amount of chitin (as a total dry weight) is the highest in crustaceans, explains why most chitosan used in commercial applications derives from these sources.[15,16] In crustacean shells, chitin is closely associated with proteins, where it acts as the bioadhesive responsible to insure the cohesion between fiber beds of the stacked laminae[17], giving structure to the crustacean and the high mechanical strength to the shell. Jeuniaux and coworkers[18] reported that the matrix in mollusk shells appears to be made of two structural units. The first unit is a high molecular weight chitinoproteic complex with no affinity to calcium, which is arranged in the form of sheets and layers. The second unit, called the mineralization matrix, is a polypeptide fraction with strong affinity for calcium, and as such, it is mostly soluble in decalcifying agents, like HCl. When $CaCO_3$ deposition occurs, chitin is trapped between mineralization matrices (Scheme 1.7).



Scheme 1.7 Mollusk shell

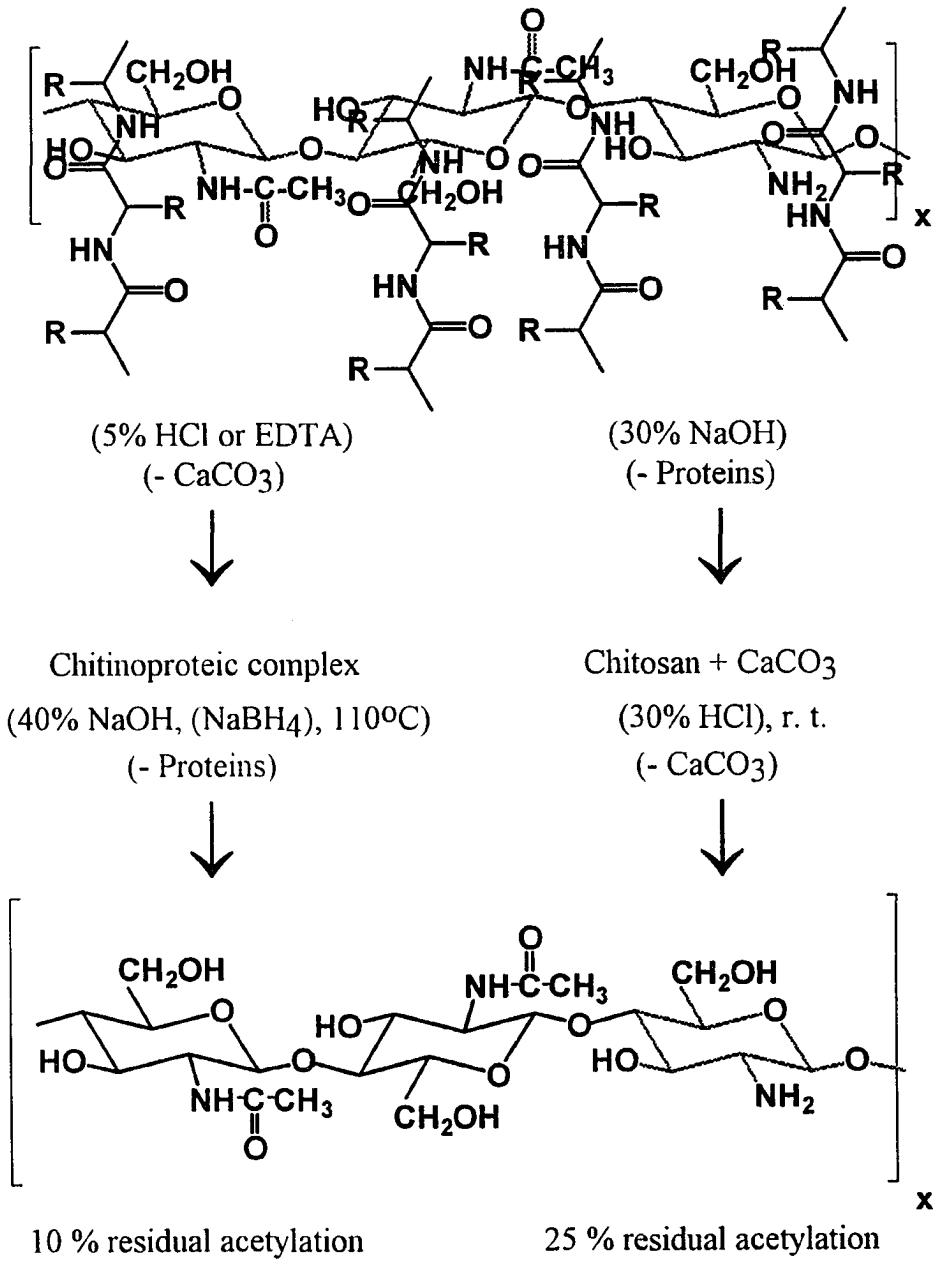
The isolation of chitosan has been achieved by different methods, including the use of enzymes,[19] bacteria,[20] and chemical treatments.[21-27] Shimahara and coworkers have isolated chitin by deproteinizing crustacean shells with a bacteria possessing a high proteolytic activity but no chitinolytic activity.[20] They did not observe deacetylation, and the crystallinity and solubility of this chitin was similar to one prepared by chemical treatments.

Nowadays, the preferred isolation method remains a chemical treatment where the demineralization of the crustacean shell is performed with HCl followed by the removal of proteins by NaOH (the order of the acid and basic treatments can be reversed). The characteristics of the final product will depend on several factors, such as the source of chitin, the concentration of reagents used, temperature of the isolation procedure and the reaction time on each step (Scheme 1.8). In 1978, Bough et al. reported one of the few studies on the effect of the alkali concentration in deacetylation, percent of deacetylation against time, and how the molecular weight and viscosity are affected as a function of time at different concentrations of NaOH.[28] In this study, they observed that when chitin was deacetylated with 50% NaOH, the percent of deacetylation and the molecular weight distribution decreased rapidly in the first hour but slowed subsequently. Moreover, as the alkali concentration was decreased from 50% to 35%, the rate at which viscosity and molecular weight distribution decreased was slowed. They studied ten different chitosan samples in flocculation of activated sludge from a vegetable processing plant and cheese whey from a dairy plant. The results showed that the effectiveness of the chitosan samples for the

two systems were opposite, prompting the authors to conclude that different grades of chitosan samples, which can be useful in different waste water treatment applications, can be manufactured by simply varying the processing conditions, especially the deacetylation step.

1.4 Enzymatic degradation of chitin/chitosan.

The wide distribution of chitin and chitosan in nature has prompted many organisms to develop hydrolytic enzymes to digest the polysaccharides either as a food source or as a defense mechanism. A classification of enzymes (chitinases) hydrolyzing chitin has been reported by Bade and Hickey.[29] They found that exochitinases are restricted to hydrolysis of one linkage per chitin chain. In the case of Streptomyces chitinase, the cleavage site is the glycosidic bond two residues distant from the non-reducing end of each chain. Endochitinases on the other hand, hydrolyze bonds randomly in the interior of the macromolecule, causing a rapid decrease in molecular weight. Enzymatic hydrolysis of chitin to acetylglucosamine can be achieved by the use of two different enzymes: chitinase and chitobiase. Chitinases are synthesized by bacteria, fungi, and digestive glands of animals whose diet include chitin, e.g. frog.[30] In order to maximize the activity of



Scheme 1.8 Isolation of Chitosan

chitinase, the chitin substrate should be free of proteins, lime and pigments. Further, it was found that the temperature of maximum activity is 37^0C, with an activity pH range between 4.5 and 7.5.[30]

Smucker presented a kinetic model for the enzymatic hydrolysis of colloidal chitin using chitinase.[31] He demonstrated that fibrils in the outside were more available for enzyme degradation, while the inside fibrils were not as readily available to the enzymes because of diffusion and crystallinity regions, resulting in different rates of hydrolysis. The mode of hydrolysis of chitooligosaccharides with chitinase and chitobiase was reported on 1982.[32] Chitinase hydrolyzed and reduced chitooligosaccharides with an endo-type mode of action, producing N,N-diacetylchitobiose and N-acetylglucosamine. Chitobiase completely decomposed chitooligosaccharides to N-acetylglucosamine.

In 1978, Fenton and coworkers[33] reported that a wide variety of microorganisms from soil and water were capable of degrading chitosan. Furthermore, chitosanases from both groups of microorganisms hydrolyzed chitosan but not chitin.They observed that the degradative action of chitosanases was dependent on the degree of acetylation of the substrate. For example, Varum et. al. have reported the extensive

degradation of a commercial sample of chitosan with stomach contents and extracts from stomach mucosa found in the atlantic salmon Salmo salar.[34] It was found that the enzyme was unable to degrade a completely deacetylated chitosan sample, indicating the need for the presence of the N-acetyl group in order for this process to occur. Most chitosanases are stable at temperatures below 50^0C, with an activity pH range between 4.5 and 7.5, showing a maximum at 5.6.[30]

Large scale production of high degree of polymerization chitooligosaccharides by the use of chitosanase obtained from the culture supernatant of Streptomyces griseus has been achieved.[35] Chitosanases showing enzymatic activity towards chitosan and glycol chitosan have been reported in the literature.[36] However, these enzymes did not show activity towards chitin, glycol chitin and carboxymethyl cellulose.

Tanaka and coworkers have reported that cellulase preparations have shown chitosanase activity as well.[37] This preparations were fractionated into two portions. The first fraction hydrolyzed high molecular weight chitosan releasing chitooligomers, however, it could not degrade low molecular weight chitosan.The susceptibility of chitosan to enzymatic hydrolysis by commercial enzymes, such as lipases,

proteases (including papain), carbohydrase, tannase and glycanases (cellulases, pectinases, a dextranase, amylase, hemicellulase and xylanase) were studied by Pantaleone et. al.[38] They found that a large number of the enzymes tested presented activity towards chitosan, even some of them had higher activities than chitinase. However, the commercial enzymes studied were impure, which might explain this unusual results.

1.5 Applications of chitosan.

Even though the research and utilization of chitosan is less advanced than cellulose, it has been attracting the attention of scientists during the last 20 years in a variety of areas, such as, biochemistry, polymer chemistry, pharmacology and medicine. This interest has risen from the applications that have been found and the potential that this material presents. During the early stages of chitosan research, the major potential applications being studied were on sludge dewatering, food processing and metal ion chelation. However, the trend nowadays is towards its use in high value products, like cosmetics, drug carriers, feed additives, semipermeable membranes and pharmaceutics.[14]

One of the most important properties of a polymer used in cosmetics is the ability to interact with negatively charged surfaces like skin and hair. When chitosan is dissolved in acids, the amino groups present in this biopolymer are protonated, giving it a cationic nature, which is responsible for the interactions between chitosan and skin and hair. Muzzarelli reviewed in 1983 the potential applications of chitosan in cosmetics.[39] This polymer has been used in the preparation of cosmetic powders,[40] nail polishes,[41] moisturizers,[42-44] hair and skin care products[43-46] and hair conditioners.[43,44,47-49]

In 1982, Gross et. al.[50] investigated several chitosan salts with the purpose of using them as film forming resins in hair products. They found different compositions with excellent properties for conditioning hair, where the concentration of chitosan salts required was lower than for conventional polymers. These solutions, which exchange water vapor without altering their properties, formed hard, non adhesive films, with good resilience. In addition, hair treated with chitosan solutions was less statically charged during brushing and combing than hair treated with conventional fixers. Another potential advantage found by these researchers was the possibility of using these salts in purely aqueous

formulations for people who can not tolerate hair fixers containing alcohol.

Muzzarelli has studied N-carboxymethyl chitosan and N-carboxybutyl chitosan for applications in cosmetics.[51] In a study to determine the skin hydration of gels and creams containing either N-carboxymethyl chitosan or hyaluronic acid, he found that the chitosan derivative based gel and cream had better hydration properties than the formulations based on hyaluronic acid for a 2 hour observation period. Furthermore, both N-carboxymethyl chitosan and N-carboxybutyl chitosan derivatives showed antibacterial action, which suggest their potential use in mouthwashes and liquid soaps.

Two different properties present in chitosan make this polymer a valuable material for the water treatment industry. First, the fact that chitosan can interact with negatively charged surfaces other than the proteins present in hair and skin, allows the use of this biopolymer for the flocculation of proteins, solids and dyes in waste waters.[52-59] Furthermore, the amino groups in chitosan can act as an electron donor ligand for the binding of metal ions, giving chitosan better chelation properties than chitin.[60]

Meyers and No reported in 1989 the use of crawfish chitosan as a coagulant for the recovery of aminoacids from seafood processing streams.[58] Their studies revealed that the concentration of suspended solids was reduced by 97%, turbidity dropped 83%, and the chemical oxygen demand decreased 45%. While the coagulated solids showed a high content of glutamic and aspartic acid, leucine, arginine and alanine, the supernatant solution had high concentrations of arginine, alanine, serine, glycine and glutamic acid.

The chelation of metal ions, such as Cu, Co, Hg, Mo,V, Cd and Pb, using chitosan has been successfully achieved.[60-70] In 1984, Hirano and coworkers used chitosan to recover uranium from rivers and lake waters in a 40-74% yield, while recovering only 3% from sea water.[14]

The derivatization of chitosan with long acyl groups has shown an improved adsorption capacity over the biopolymer itself.[71] In this experiment, nonanoylated chitosans increased the Cu^{2+} uptake by 23%, with the maximum capacity at a degree of nonanoylation of less than 0.1.

Domard and coworkers studied in 1989 the adsorption of chitosan and N-trimethylchitosan chloride on kaolin.[153] They concluded in this study that the best conditions can be obtained with a polymer fully

deacetylated with high molecular weight. Moreover, the quaternized derivative did show flocculation activity as well, but degradation that occurred during its synthesis caused a lower molecular weight, and lower flocculation activity than chitosan was observed.

The biomedical applications of chitosan are attributed to its biological properties, which are compatibility with most living systems and biodegradability. It has shown hemostatic, bacteriostatic, fungistatic, spermicidal. antitumor, antithrombogenic, anticholesteremic and immunoadjuvant properties,[14,72] while its sulfated derivative presents blood anticoagulant properties.[14] Chitosan is already available in a variety of forms depending upon the medical application targeted, i.e. powder, paste, solution, bandages, sutures, wound dressing, etc.[14,72,73]

The use of chitosan and its derivatives in areas such as biotechnology and agriculture has been well documented.[14,73] This biopolymer has been proved to be useful in cell and enzyme immobilization, controlled release systems, hydroponic fertilizer, to improve soil properties, and to coat seeds, resulting in more productive crops.

1.6 Objective of the present study.

We decided to initiate our work with chitin/chitosan in order to try to solve several problems encountered in the state. Thanks to the productivity of Louisiana's swamps, marshes and sea coast, a steady supply of raw material for the production of chitosan can be obtained. Moreover, when shrimp and crawfish are processed in the state's seafood industry, their shells are usually considered a waste, and they are thrown back into the rivers, polluting the water. Therefore, we believe that by using this byproduct as a raw material for a commercial product, we could help to solve an environmental problem, while if the materials that we obtain show interesting properties, it might be the basis for creating a new industry for the state of Louisiana.

CHAPTER TWO

SYNTHESIS AND SPECTROSCOPIC CHARACTERIZATION OF CHITOSAN DERIVATIVES

2.1 Attempted synthesis of chitin derivatives.

Since the alkaline hydrolysis of chitin is the highest cost step in production of chitosan, we attempted to by-pass that step by using chitin as our substrate. Our first attempts to work with the chitin/chitosan system involved the reaction of a chitin sample with a 46% deacetylation (determined by IR[74]) with p-toluenesulfonyl chloride (tosyl chloride), which had been reported previously by Kurita et. al.[75] In this study, tosylation of chitin was first attempted on chitin dispersed in pyridine/DMSO, but no appreciable extent of reaction was detected. However, when aqueous alkali chitin was reacted interfacially with a chloroform solution of tosyl chloride, the reaction proceeded smoothly. The product was then treated with sodium iodide in DMSO. The authors obtained tosyl chitin and iodo chitin which were soluble in polar organic solvents such as, DMF, NMP, DMSO. We attempted to react chitin (first in a 12% NaOH solution, then in a 50% NaOH solution) with a solution of tosyl chloride in chloroform for 1 hour at 0°C (to disrupt

crystallinity), followed by 4 hours of reaction at room temperature with no success. Even running the reaction for an additional 2 hours at reflux did not yield the desired tosyl chitin. The use of phase transfer catalysts like tetramethyl-ammonium chloride or sodium dodecyl sulfate did not render the target product either. Since chitin was swelling in basic conditions using DMSO as a solvent, we attempted to solubilize chitin (in a 50% NaOH solution) by reacting it with carbon disulfide (xanthation reaction) in DMSO. However, chitin did not react and the desired chitin xanthate was not obtained. The failure to duplicate the chitin reactions reported in the literature was attributed to differences in both the molecular weight and the degree of deacetylation between chitin samples.

2.2 Determination of the degree of deacetylation of chitosan.

One of the most important parameters to determine in a sample of chitosan is the degree of deacetylation. This characteristic is directly related to the hydrogen bonding existing in this biopolymer, thus affecting the structure, solubility and ultimately its reactivity. The degree of deacetylation has been determined by titration[76] , dye adsorption[77], UV[60,78], VIS[79], IR,[27,74,80-83] and NMR spectroscopy.[84,85] We determined

the degree of deacetylation of chitin and chitosan samples by two different methods, IR and NMR. Determination of deacetylation by IR spectroscopy is a more practical approach because it can be applied to insoluble chitin samples.

2.2.1 IR method.

In 1985, Roberts et. al.[74] evaluated the use of IR spectroscopy in chitosan samples ranging from 14% to 72% residual N-acetyl. They observed, by comparing this technique with the titration of chitosan hydrobromide salts and with the UV spectroscopic determination of N-salicylidene-chitosan (a Schiff base) formation, that there was a linear correlation between the %N-acetyl determined by IR and the same parameter determined by chemical techniques. Moreover, their results indicated that this technique was relatively insensitive to small amounts of adsorbed water.

We calculated the degree of deacetylation by determining the absorption of the amide band at around 1650 cm^{-1} and comparing it to the absorption of the hydroxyl band at around 3450 cm^{-1}, which is used as an internal reference (Figure 2.1). Once this data is obtained, the

residual acetyl groups can be calculated by the use of the following formulas:

$$\% \text{ N-acetyl} = (A_{1650}/A_{3450})(100/1.33)$$

$$\% \text{ Deacetylation} = 100 - \% \text{ N-acetyl}$$

2.2.2 NMR method.

Domard and coworkers[84] were the first researchers to determine the ^{1}H NMR of chitosan, as well as the ^{1}H-^{1}H and ^{13}C-^{1}H correlations, thus allowing the determination of the degree of deacetylation by this method. This technique permitted them not only to characterize chitosan, but to study and determine the position of the substituents in the N-trimethyl chloride[84] and the O,N-carboxymethyl derivatives.[85] Furthermore, this technique has been used by Rinaudo and coworkers to assign the chemical shifts of ^{1}H and ^{13}C NMR signals for chitosan,[85] as well as the degree of deacetylation. Similar to the IR method, this technique was adapted to determine the degree of deacetylation in our chitosan sample.

In the NMR spectrum (Figure 2.2), the N-acetyl peak appears at 2.05 ppm, while H-1 and H-2 appear around 5.0 ppm and 3.2 ppm respectively, which are used as internal references. The remaining

hydrogens, 3, 4, 5 and 6 appear as a clustered signal between 3.5 and 4.2 ppm. The calculation of residual N-acetyl groups is achieved by dividing the integral of the N-acetyl signal at 2.05 ppm by 3 (3 hydrogens per N-acetyl group), and the result of this calculation is divided by the integral of H-1 (the H-2 signal can also be used), as exemplified by the following formulas:

$$\% \text{ N-acetyl} = (I_{2.05}/3)/(I_{5.0}) * 100$$

$$\% \text{ Deacetylation} = 100 - \% \text{ N-acetyl}$$

2.3 Synthesis of chitosan derivatives.

Since all of our results trying to use chitin as a starting material had been negative, we decided to work with chitosan. As the degree of deacetylation plays a major role in this system, special attention was paid to its determination. In the sample of chitosan used in this research, we determined the degree of deacetylation to be 79% by IR[74] (Figure 2.1) and 83% by NMR[85] (Figure 2.2); the manufacturer reported 77% using a completely different method.

2.3.1 Regeneration of chitosan.

The substitution of chitosan, similar to other polysaccharides, is determined by the availability of the functional groups to the reagents;

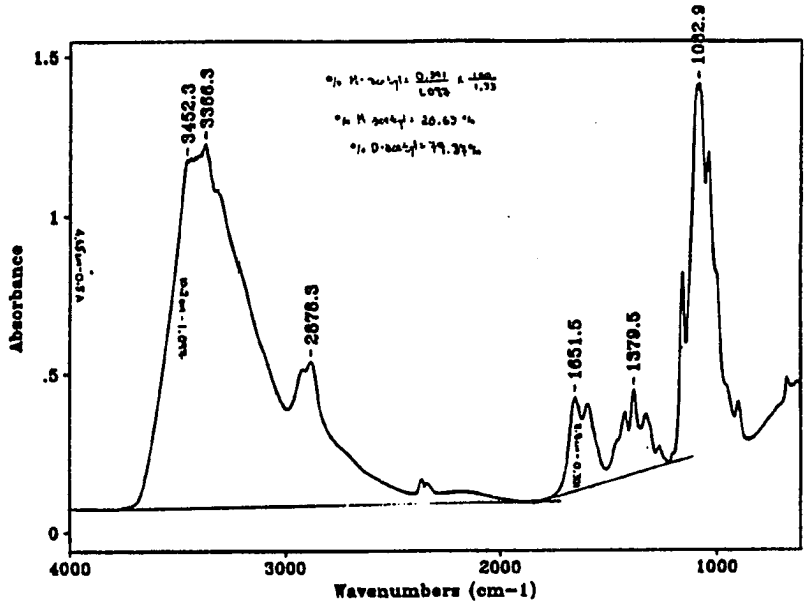


Figure 2.1 Determination of the degree of deacetylation of chitosan by FTIR.

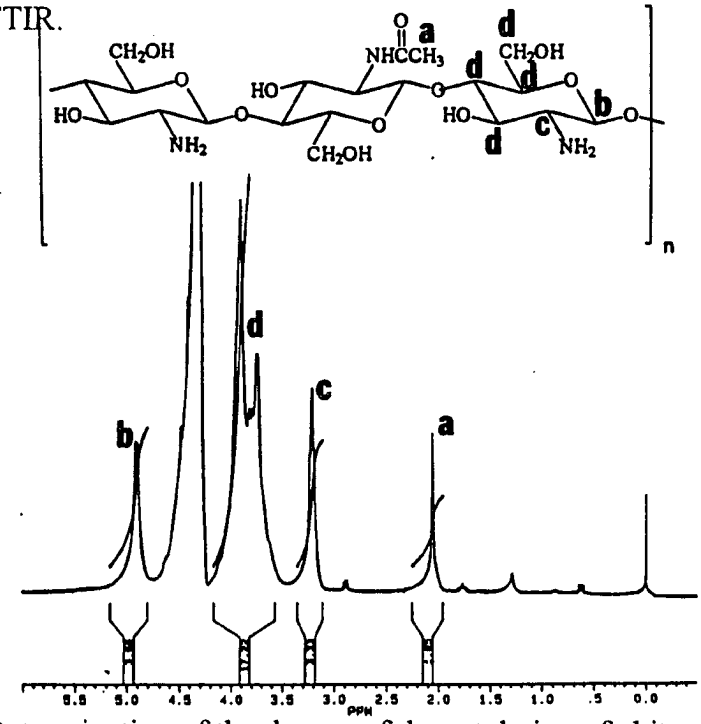


Figure 2.2 Determination of the degree of deacetylation of chitosan by NMR.

this depends on the diffusion rates of the reagents into the substrate, degree of deacetylation, the nature of the chitosan being used, and the pretreatment given to the biopolymer prior to the reaction. To improve the reactivity of intractable polysaccharides, several methods have been developed; among the methods most commonly used are, solvent exchange, inclusion techniques, and the incorporation of surface active or complexing agents (zinc oxide, urea, etc.). Another technique frequently used, in particular for etherifications, involves the treatment of polysaccharides with high concentrations of base in situ.[86]

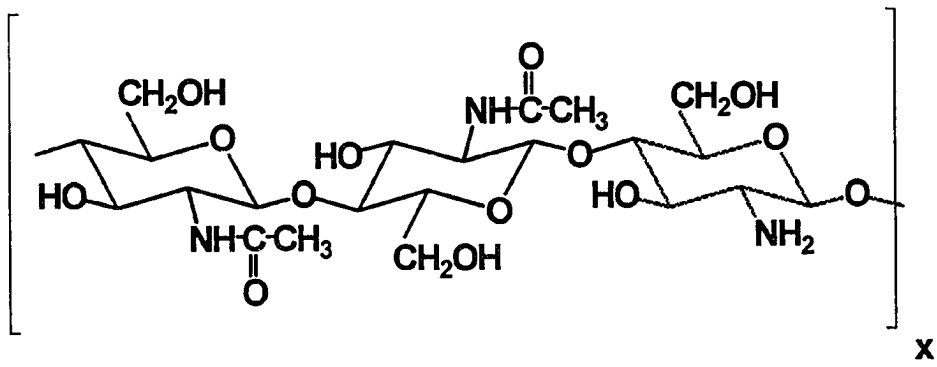
In order to obtain more homogeneous reactions, and therefore products with improved solubilities, we decided to regenerate chitosan, and thus disrupt the crystallinity of the biopolymer prior to subsequent reactions. Similar processes where chitosan has been dissolved with HCl followed by reprecipitation with NaOH,[87-89] and dissolved in organic acids and organic diluents followed by reaction of the biopolymer have been reported.[90,91] The regeneration of chitosan approach that we employ consists of dissolving the polysaccharide in 15% Acetic acid for 30 min. followed by reprecipitation with 15% $NaHCO_3$ (Scheme 2.1). Then, the regenerated biopolymer was reacted with either epoxides or

acrylonitrile. This pretreatment has proven to be a very effective method for disrupting the crystallinity present in the biopolymer, increasing the number of reactive sites available to reagents; which has resulted in the reduction of reaction time for the synthesis of various derivatives, improved solubilities of the products, and has permitted us to use water as the solvent instead of isopropyl alcohol.

2.3.2 Reaction of chitosan with epoxides.

The nonionic hydroxyalkylcellulose ether polymers, hydroxyethyl cellulose and hydroxypropyl cellulose, are materials widely used in industries such as pharmaceuticals, textiles, paper, adhesives, paints, food and cosmetics, where applications for thickening, lubricating, protective colloid, stabilizer and binding properties are required.[92] Although chitosan analogues also belong to the same carbohydrate family, the potential for creating a positively charged material should create a substantial different activity and enhance their applications as specialty water soluble resins.

A few patents on the synthesis and characterization of hydroxyalkylchitosan ether polymers (hydroxyethyl, hydroxypropyl,

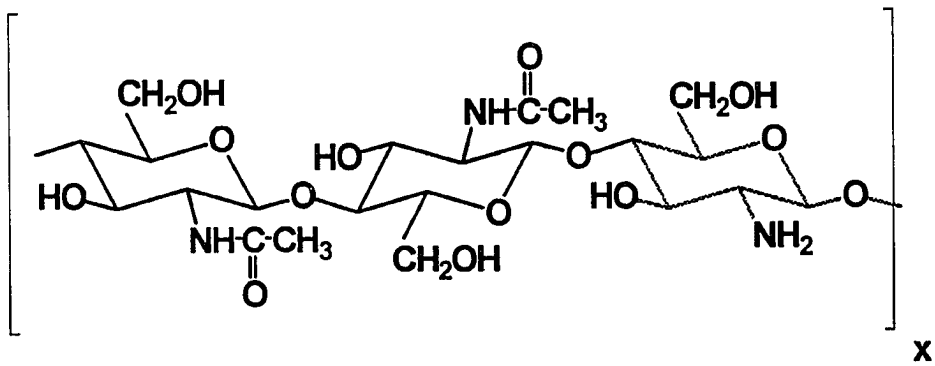


flakes

↓ 15% HOAc

Viscous solution

↓ 15% $NaHCO_3$



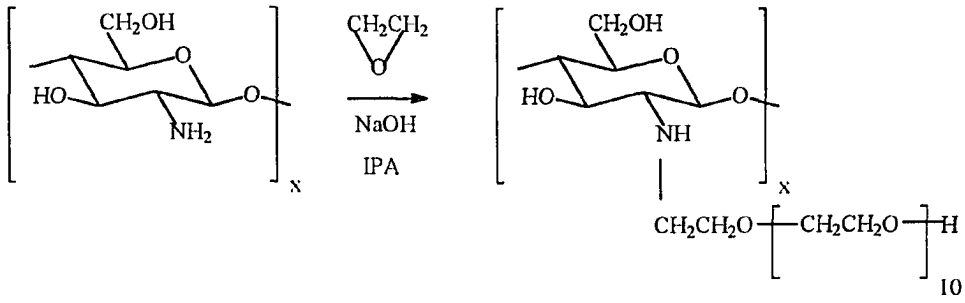
swollen

Scheme 2.1 Regeneration of chitosan.

dihydroxypropyl, glycidyltrimethylammonium chloride, and 2-hydroxypropyl trimethylammonium chloride) have been reported recently by several researchers mainly in the industry.[87-89,93-109] Further, some of these patents are targeted towards employing these derivatives in commercial applications. For example, hydroxyethyl chitosan and its derivatives have been used in cosmetic formulations,[89,102] as well as in diapers and sanitary napkins.[104] Hydroxypropyl chitosan and its derivatives have been employed mostly to prepare cosmetic formulations;[88,99,100,105,107,110,111] it has been studied also for sustained release of water soluble drugs.[106]

Even though these compounds have already been synthesized, most of the work produced to date involves the use of high concentrations of base, or high pressure, or high temperature, which can degrade the polymer chain. Our objective was to design synthetic methodologies to obtain these derivatives under milder conditions in order to avoid the degradation of chitosan.

2.3.2.1 Hydroxyethyl chitosan (Hech).



Scheme 2.2 Synthesis of hydroxyethyl chitosan.

We have synthesized N-hydroxyethylchitosan under basic heterogeneous conditions. In this process, chitosan flakes were first swollen in a 15% NaOH solution using isopropyl alcohol as the solvent, followed by addition of ethylene oxide to the reaction flask. The reaction mixture became slightly yellow and viscous, however, when it was neutralized, the color decreased in intensity while the viscosity dropped. The structure of the product, which is water soluble, has been confirmed by FTIR and NMR. The FTIR analysis (Figure 2.3) shows a change in the OH band at 3400 cm^{-1} (compared to chitosan), a sharp increase in the CH stretching region at around 2900 cm^{-1} and an ether band at 1100 cm^{-1}. Additionally, the NMR spectrum (Figure 2.4) shows the presence of the methylene groups in the ether chain at 3.6 ppm, overlapping with

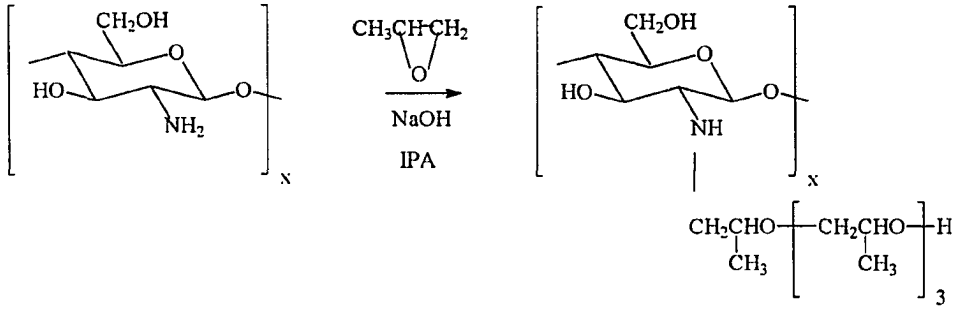
several signals present in chitosan. Further confirmation of the structure is seen with the upfield shift of H-1 from 4.9 ppm to 4.4 ppm, and the upfield shift of H-2 from 3.2 ppm to the 2.5-3.0 ppm region. Results from the NMR spectrum indicate a D.S.= 1.4 and a M.S.= 11.1.

2.3.2.2 Regenerated hydroxyethyl chitosan (R-Hech).

Hydroxyethyl chitosan synthesized from regenerated chitosan showed improved solubility properties when compared with the same product obtained by the more heterogeneous process already described. The reaction between fresh swollen chitosan and ethylene oxide was run in a 15% NaOH solution using water as the solvent instead of isopropyl alcohol. As the reaction proceeded, more water was added to the system causing the reaction mixture to clear up immediately, thus obtaining a homogeneous reaction for 24 hours. To conclude, this methodology allowed us to avoid the chitosan swelling step used previously, which was taking 24 hours; and therefore, a reduced reaction time was needed to obtain a more homogeneous process while water was used as the solvent. The analysis by IR (Figure 2.5) showed less absorption for the CH band when compared to the OH band, suggesting a lower D. S. or M. S., or both, when compared to Hech. Furthermore, the NMR

spectrum (Figure 2.6) confirmed this observation, which indicate a D. S.= 1.5 and M. S.= 4.4.

2.3.2.3 Hydroxypropyl chitosan (Hpch).



Scheme 2.3 Synthesis of hydroxypropyl chitosan.

Similar to N-hydroxyethylchitosan, N-hydroxypropylchitosan was obtained by the use of two different methodologies. The more heterogeneous process involved the swelling of chitosan at room temperature in a reaction mixture containing 15% NaOH, propylene oxide, and isopropyl alcohol as the solvent, followed by an increase in temperature to 50°C, where the temperature was held for 48 hours. During the course of the reaction, a transparent film formed on the walls of the reaction flask, and the reaction mixture became yellow and viscous, but when it was neutralized, it turned clear while the viscosity decreased.

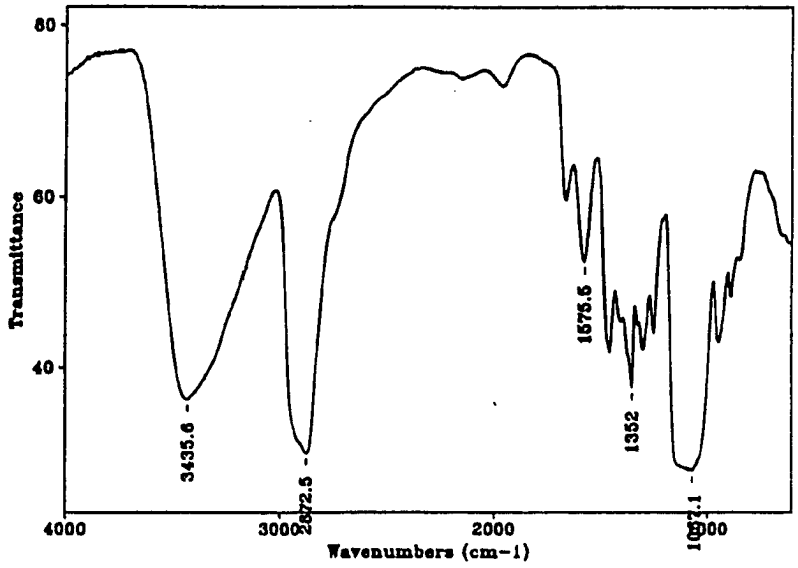


Figure 2.3 FTIR of hydroxyethyl chitosan.

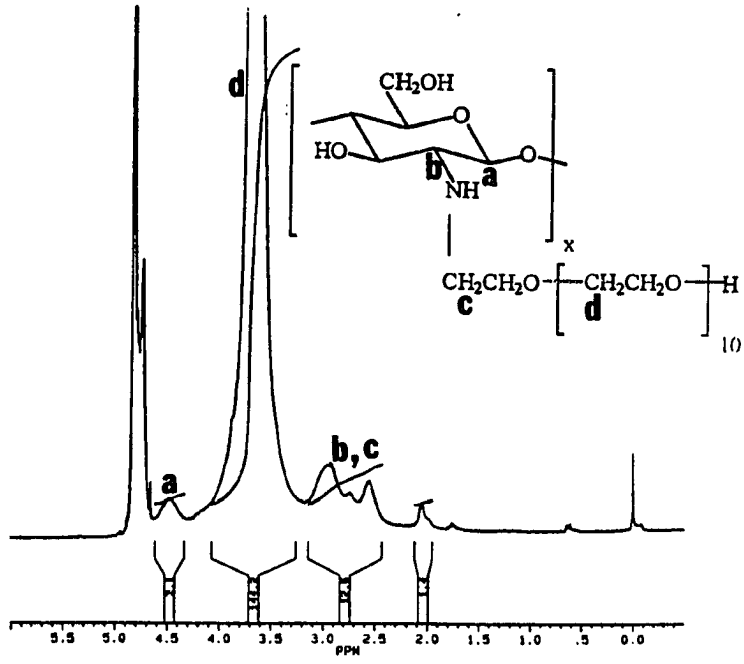


Figure 2.4 NMR of hydroxyethyl chitosan.

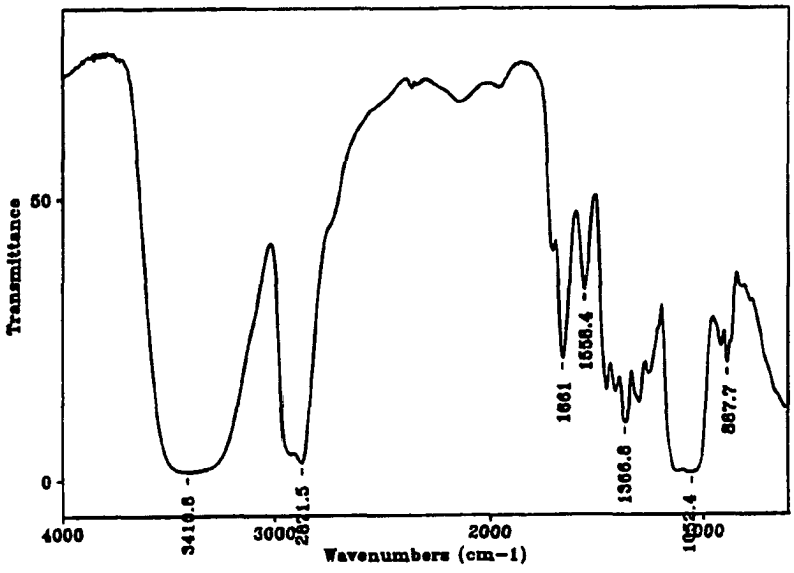


Figure 2.5 FTIR of R-hydroxyethyl chitosan.

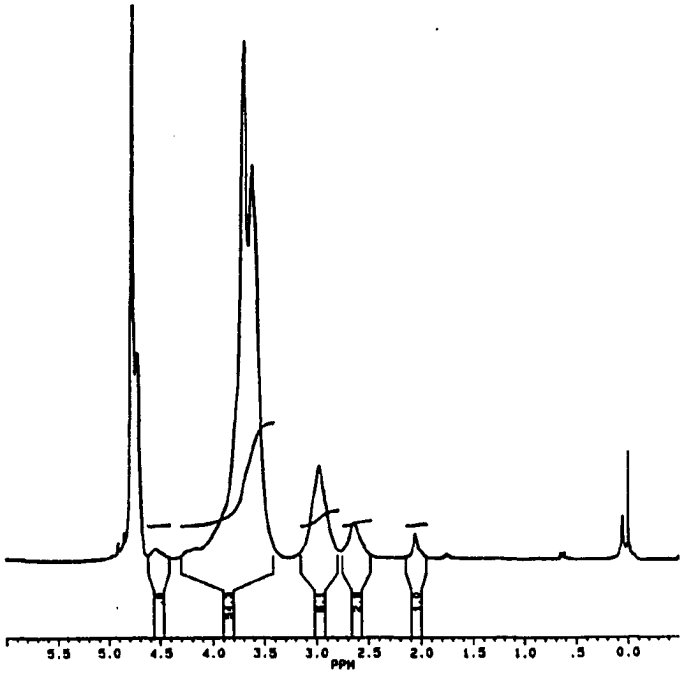


Figure 2.6 NMR of R-hydroxyethyl chitosan.

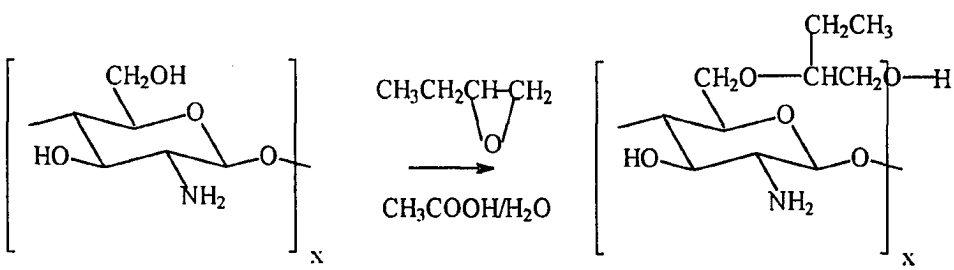
The structure of the product has been confirmed by FTIR and NMR spectroscopy. One observes in the IR spectrum (Figure 2.7): a change in the OH band at 3400 cm^{-1} (compared to chitosan), an increase in the CH stretch at 2900 cm^{-1} and the appearance of the ether band at 1100 cm^{-1}. In the NMR spectrum (Figure 2.8), we can see upfield shifts for H-1 (from 4.9 to 4.4 ppm) and H-2 (from 3.2 ppm to 2.5-3.0 ppm region), while the methyl peak appears at around 1.2 ppm. From the NMR spectrum we have determined a D.S.= 1 and M.S.= 4.

2.3.2.4 Regenerated hydroxypropyl chitosan. (R-Hpch).

Hydroxypropyl chitosan was also obtained using regenerated chitosan in a more homogeneous process. In this synthesis, fresh swollen chitosan was slurried in propylene oxide and 15% NaOH solution. After 4 hours at room temperature, the addition of water to the slurry yielded a clear solution which became slightly yellow and viscous as the reaction proceeded, when the reaction was neutralized, it turned clear as the viscosity dropped. Using this strategy permitted us to avoid the chitosan swelling step used previously, which was taking 24 hours, as well as the reduction of the total reaction time (from 72 hours to 24 hours); while obtaining a more homogeneous process where water was used as the

solvent. The data obtained from IR (Figure 2.9) is consistent with the data obtained for Hpch. NMR analysis (Figure 2.10) resulted in a D. S.= 2.2 and M. S.= 5.6. Although the solubility of this polymer synthesized by this more homogeneous process (R-Hpch) is as good as the product obtained from the more heterogeneous process (Hpch), it seems that the former compound needs more time to dissolve when placed in water.

2.3.2.5 Hydroxybutyl chitosan (Hbch).



Scheme 2.4 Synthesis of hydroxybutyl chitosan.

After obtaining successful reactions with ethylene oxide and propylene oxide, we tried to react chitosan with 1,2-butylene oxide and styrene oxide under similar conditions in an effort to make a more hydrophobic, organic soluble derivative. However, products with detectable substitution could not be produced. Since we know that epoxides can react under acidic (S_N^1) or basic (S_N^2) conditions due to the ring strain, we decided to run some experiments using dilute acetic acid

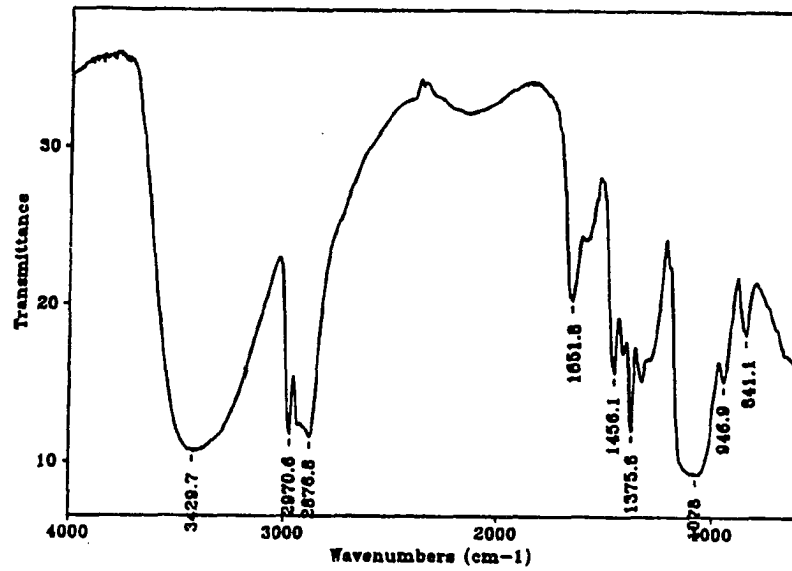


Figure 2.7 FTIR of hydroxypropyl chitosan.

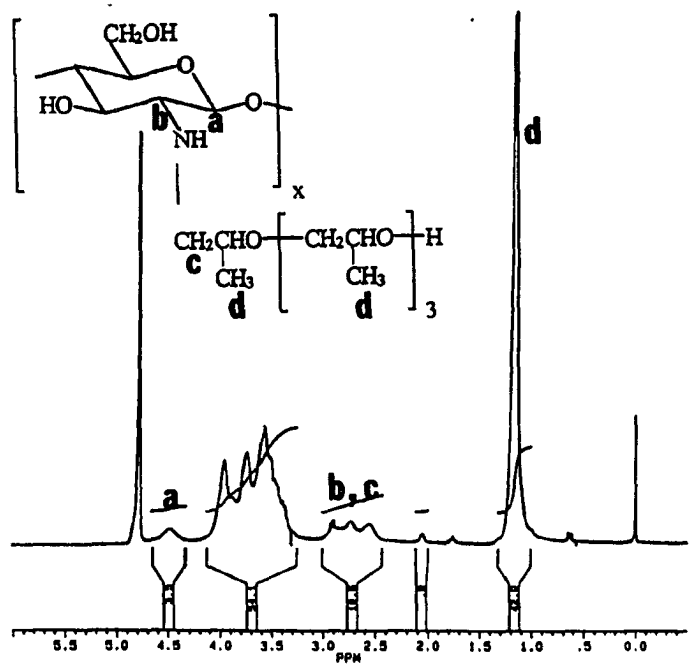


Figure 2.8 NMR of hydroxypropyl chitosan.

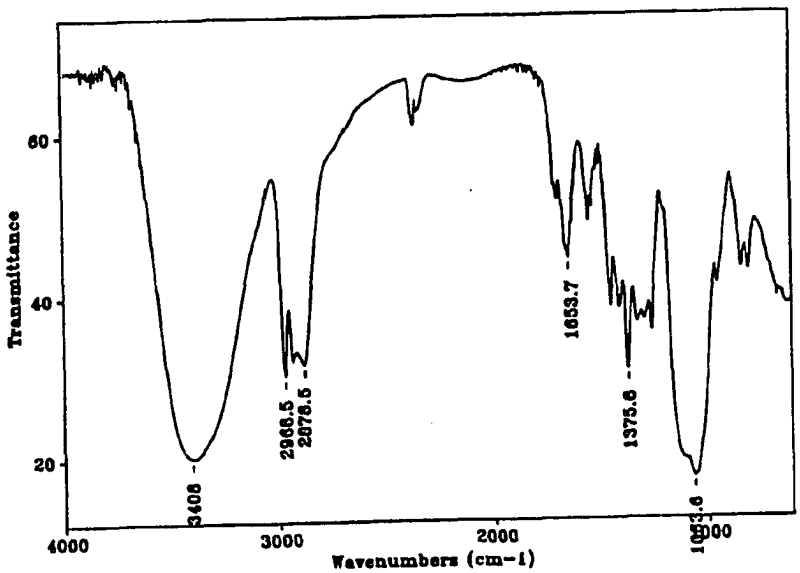


Figure 2.9 FTIR of R-hydroxypropyl chitosan.

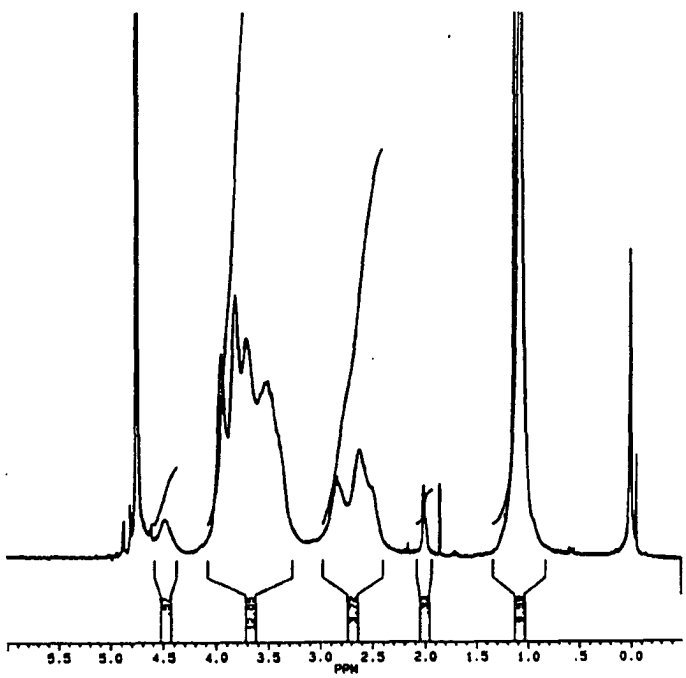


Figure 2.10 NMR of R-hydroxypropyl chitosan.

as the solvent and the catalyst, while at the same time taking advantage of the solubility of chitosan in this medium.

Chitosan was first dissolved in 15% acetic acid, followed by the addition of 1,2-epoxybutane and a small amount of methanol to make the two phases miscible. Then, it was kept at 90°C for 3 days. Even though the conditions were harsh, the reaction did not take place to a great extent. The IR spectrum (Figure 2.11) indicated a slight increase (compared to chitosan) in the OH band at around 3400 cm^{-1}, as well as in the CH stretching region at about 2900 cm^{-1}. The NMR spectrum (Figure 2.12) indicated a D. S. = 0.1., confirming the data obtained by IR regarding a small degree of reaction. It is observed in the NMR that the H-2 signal did not move upfield, as it did in Hech and Hpch, it stayed at 3.2 ppm, suggesting reaction at position 6 instead of 2. However, when compared to the H-2 signal, 26% of H-1 did move upfield to 4.6 ppm, which is due to the degradation of chitosan. Further, the methyl group appears at 0.9 ppm, while the methylene signal is at 1.5 ppm.

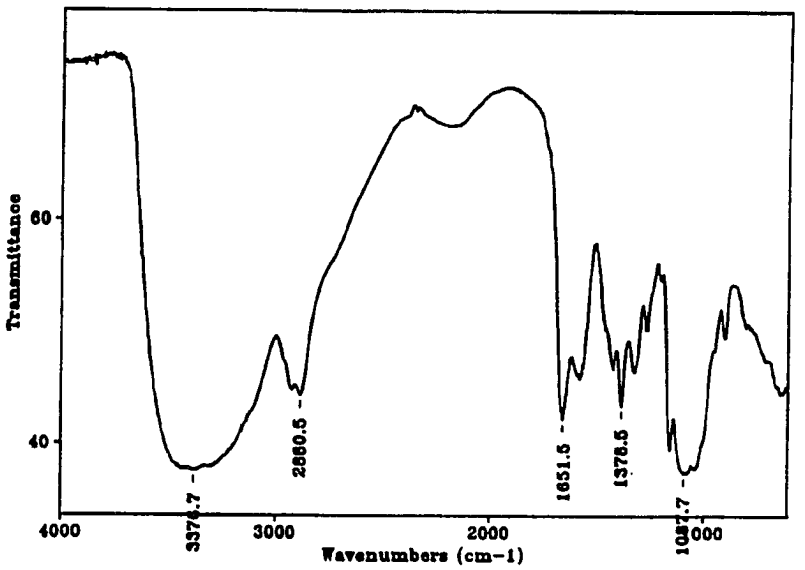


Figure 2.11 FTIR of hydroxybutyl chitosan.

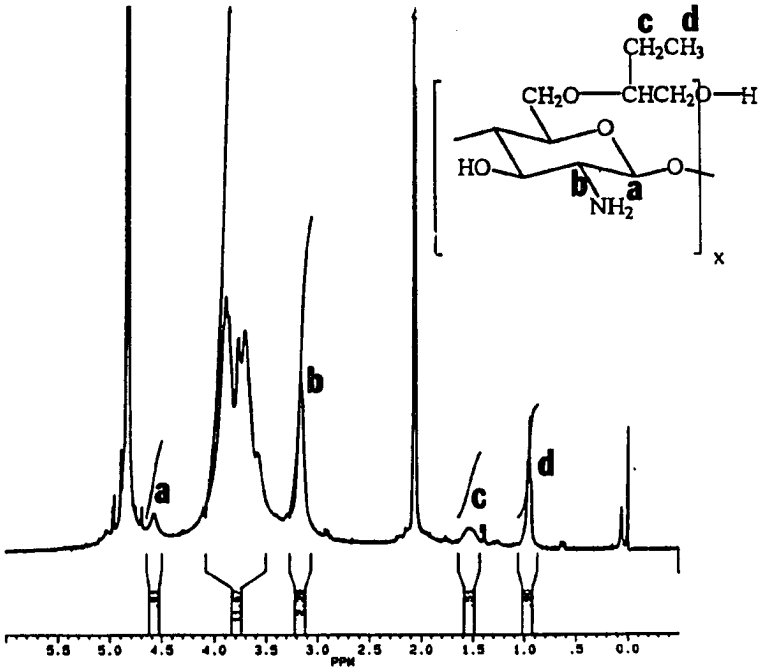
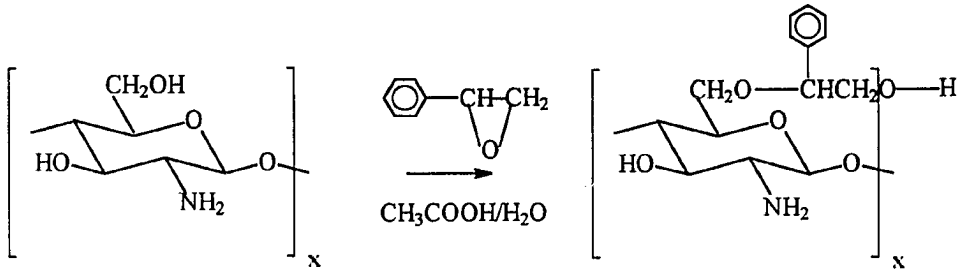


Figure 2.12 NMR of hydroxybutyl chitosan.

2.3.2.6 Hydroxy(2-phenyl)ethyl chitosan (Hphch).



Scheme 2.5 Synthesis of hydroxy(2-phenyl)ethyl chitosan.

The method followed to react chitosan with styrene oxide consisted of exactly the same steps and reaction conditions utilized to obtain hydroxybutyl chitosan.. The IR spectrum (Figure 2.13) showed very little increase (compared to chitosan), if any, in the OH, CH and ether bands. The NMR spectrum (Figure 2.14) showed the same features observed for hydroxybutyl chitosan. The H-2 signal did not move upfield, suggesting reaction at position 6 instead of 2; but a small amount of H-1 moved upfield to 4.5 ppm (53% of H-2) due to chitosan degradation. The aromatic signals appeared between 7.0 and 8.0 ppm, and the two methylenes present in styrene appeared between 2.8 and 3.1 ppm. The D. S. = 0.5 indicates a product with low degree of substitution.

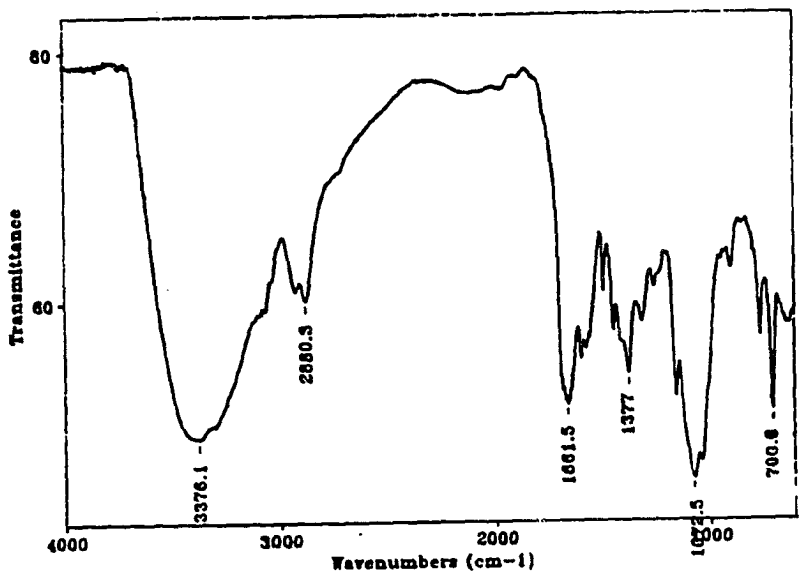


Figure 2.13 FTIR of hydroxy(2-phenyl)ethyl chitosan.

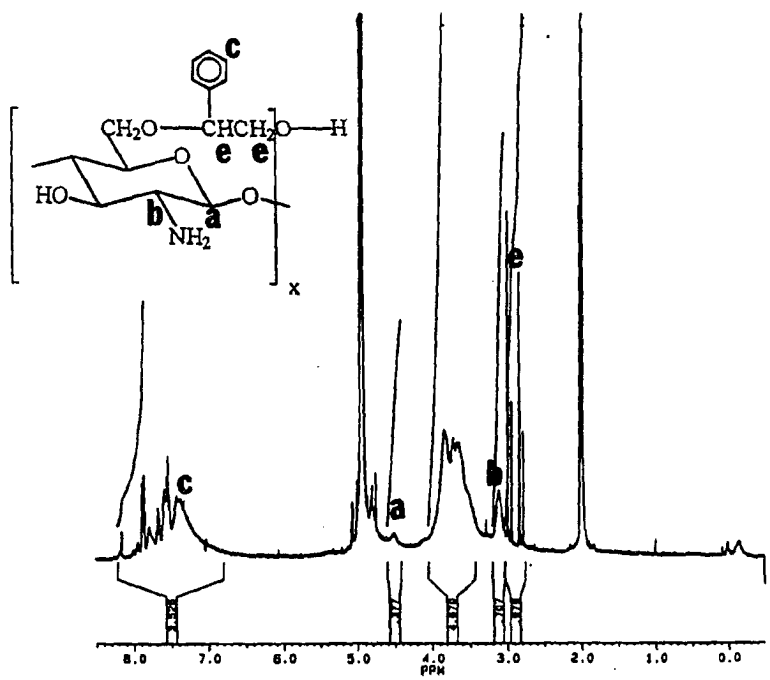
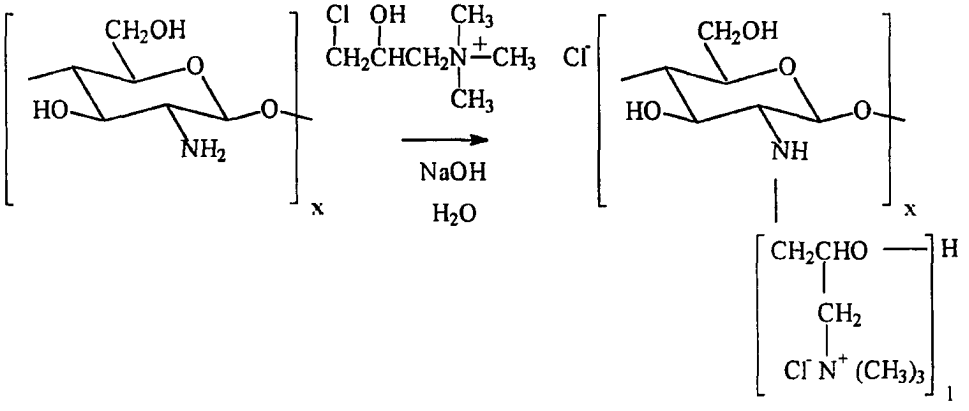


Figure 2.14 NMR of hydroxy(2-phenyl)ethyl chitosan.

2.3.2.7 2-Hydroxypropyl trimethylammonium chitosan chloride. (Chitosan-quat 188 or R-ch-quat 188).



Scheme 2.6 Synthesis of chitosan-quat 188.

Quat 188 is an aqueous solution of N-(3-chloro-2-hydroxypropyl) trimethylammonium chloride salt manufactured by The Dow Chemical Co. This salt has been used to introduce a cationic moiety to starch and guar gum, increasing their affinity for anionic materials, which renders biopolymers that are valuable as flocculants.[112] When this salt is treated with a strong base, the alcohol group will attack the neighboring carbon containing chlorine, which is a good leaving group, forming 2,3-epoxypropyltrimethylammonium chloride. This epoxide can then further react, similar to ethylene oxide, propylene oxide, 1,2-epoxybutane and styrene oxide, with alcohol or amine groups.

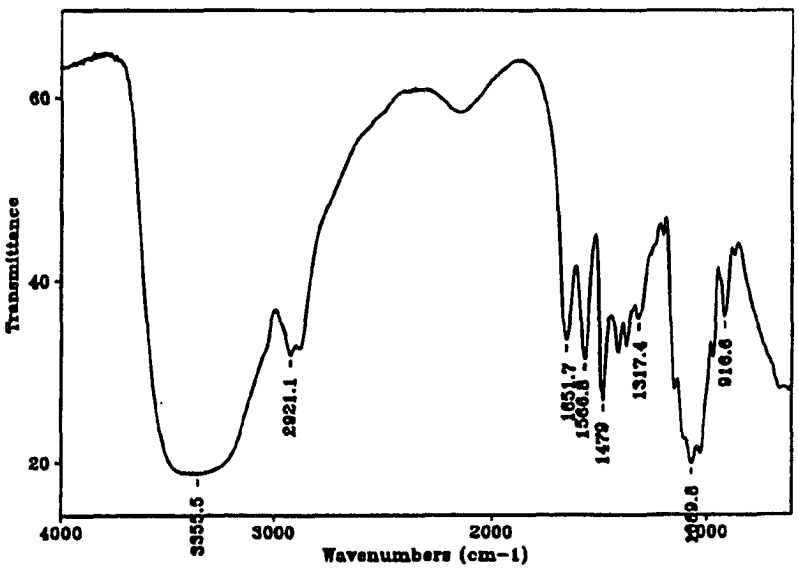


Figure 2.15 FTIR of R-chitosan-quat 188.

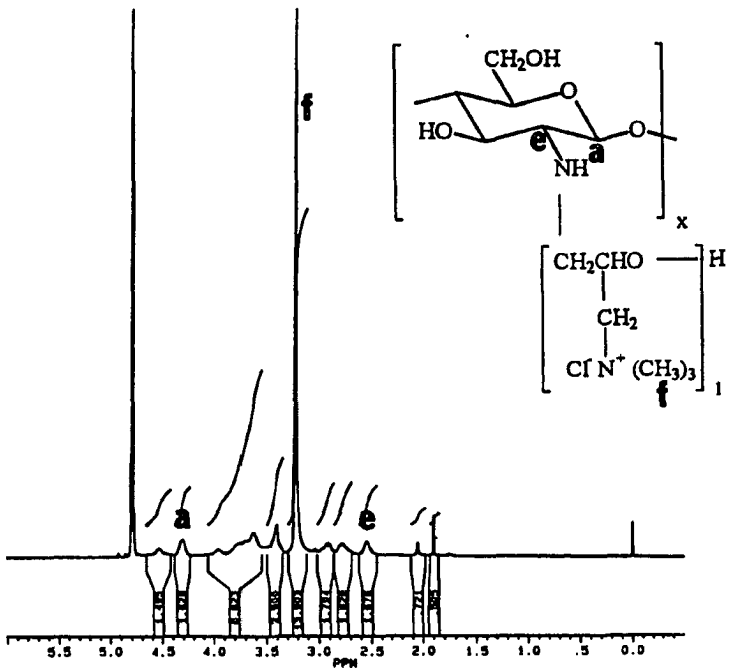


Figure 2.16 NMR of R-chitosan-quat 188.

The synthesis of chitosan-quat 188 was achieved by a basic catalyzed procedure resembling the conditions used for the synthesis of R-Hpch. To obtain this derivative, fresh regenerated chitosan was first slurried in quat 188 and 15% NaOH solution, followed by addition of water and an increase in temperature to 50°C, which gave a clear and viscous solution that was reacted for 24 hours. The IR spectrum (Figure 2.15) did show an increase in the hydrogen bonding band for the OH (when compared to chitosan) at around 3400 cm^{-1}; however, the CH and ether bands did not increase dramatically. This result was confirmed with the NMR spectrum (Figure 2.16), where calculations indicate a D. S.= 1.1 and M. S.= 0.9. It is observed that the H-1 peak appears at 4.3 ppm, the H-2 appears around 2.55 ppm, and the CH_3 signal from the quaternary ammmonium salt shows around 3.2 ppm.

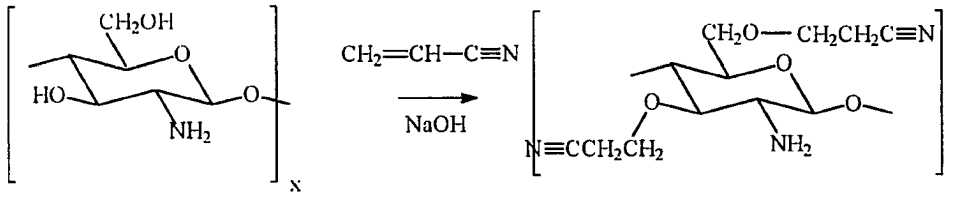
2.3.3 Reaction of chitosan with acrylonitrile.

The ease by which Michael addition reactions occur at primary amines, suggested the possibility of a reaction between chitosan and acrylonitrile, with the possibility of finding some solubility of the cyanoethylated chitosan in polar organic solvents. Furthermore, the nitrile moiety presents the potential of undergoing reduction and

hydrolysis reactions, which would yield chitosan derivatives with different degrees of hydrolysis.

There is very limited information on the cyanoethylation of chitosan and its solubility,[113-115] which has been limited to formic acid. Our objective was to obtain water and polar organic soluble or swollen products through the synthesis of cyanoethyl chitosan and some of its potential derivatives.

2.3.3.1 Cyanoethyl chitosan (Cech).

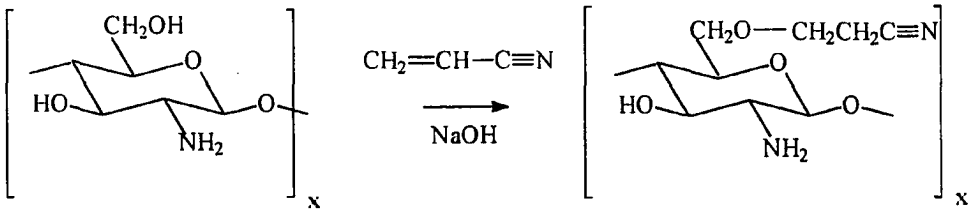


Scheme 2.7 Synthesis of cyanoethyl chitosan.

Our first attempts at modification of chitosan by Michael addition involved the heterogeneous reaction of chitosan flakes with acrylonitrile in a 15% NaOH solution for 24 hours. The flakes turned slightly white during the course of the reaction, but complete dissolution was not achieved. We can conclude from the IR spectrum (Figure 2.17) that reaction took place effectively as indicated by the presence of the band at 2250 cm^{-1} characteristic of the nitrile group. Substitution is confirmed

by the NMR spectrum (Figure 2.18), showing the two new methylene peaks added to the polymer chain at 3.8 ppm and 2.8 ppm. Additionally, the H-1 peak did not move upfield, it remained at 4.8 ppm, while H-2 stayed at 3.2 ppm; this result, combined with a D. S. = 2 (calculated from NMR), suggests that reaction took place at position 6 and 3. However, the product was soluble only in acetic acid.

2.3.3.2 Regenerated cyanoethyl chitosan (R-Cech).

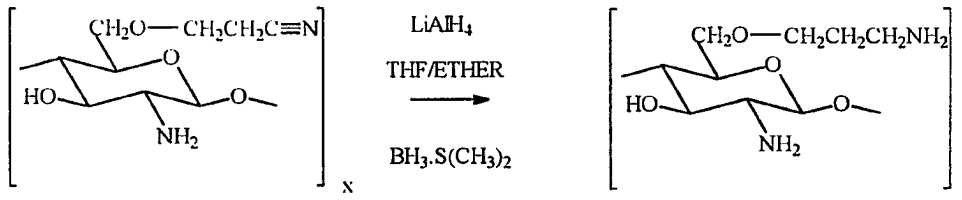


Scheme 2.8 Synthesis of regenerated cyanoethyl chitosan.

The technique of regenerating chitosan before reaction with acrylonitrile was also employed to synthesize this compound. In this process, fresh regenerated chitosan was slurried with acrylonitrile in a 15% NaOH for 4 hours at room temperature, followed by addition of water, which gave immediately a yellow viscous solution that was reacted for an additional hour. The product obtained by this method swells in solvents such as DMSO, toluene, water, and diluted NaOH. The IR (Figure 2.19) and NMR (Figure 2.20) spectra showed the same

features seen in Cech, which was produced by the more heterogeneous method. However, the absorption of the nitrile peak in the IR spectrum was lower for R-Cech than for Cech, when compared to the absorptions of the OH and CH bands. The analysis of the NMR spectrum revealed a lower D. S. = 1, thus explaining the lower absorption of the nitrile peak. We believe that reaction took place at the most reactive primary alcohol group at position 6 rather than in position 3, where there is a less reactive secondary alcohol group.

2.3.3.3 Aminopropyl chitosan (Apch).



Scheme 2.9 Synthesis of aminopropyl chitosan.

Our interest in synthesizing this derivative was originated from the idea that aminopropyl chitosan could show better chelating properties than chitosan due to the presence of two amino groups rather than one. Although there is not work reported in the literature for chitosan, its cellulose analogue has already been prepared.[116]

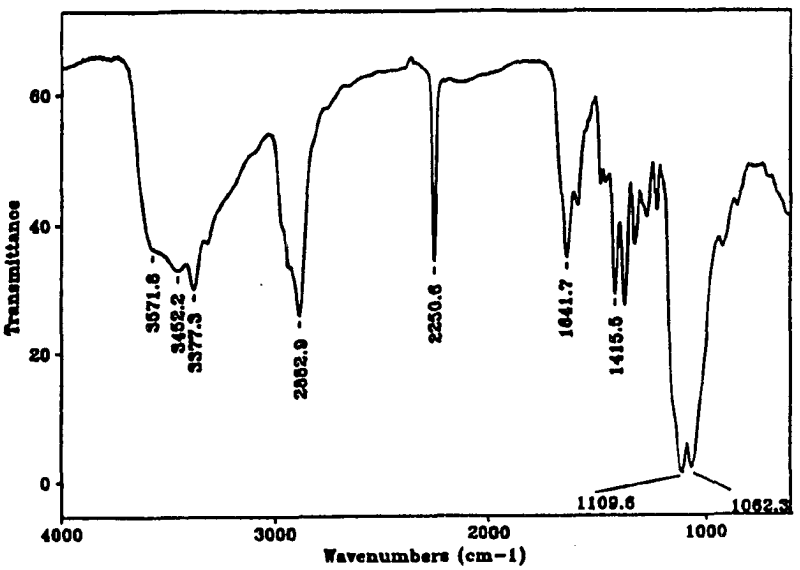


Figure 2.17 FTIR of cyanoethyl chitosan.

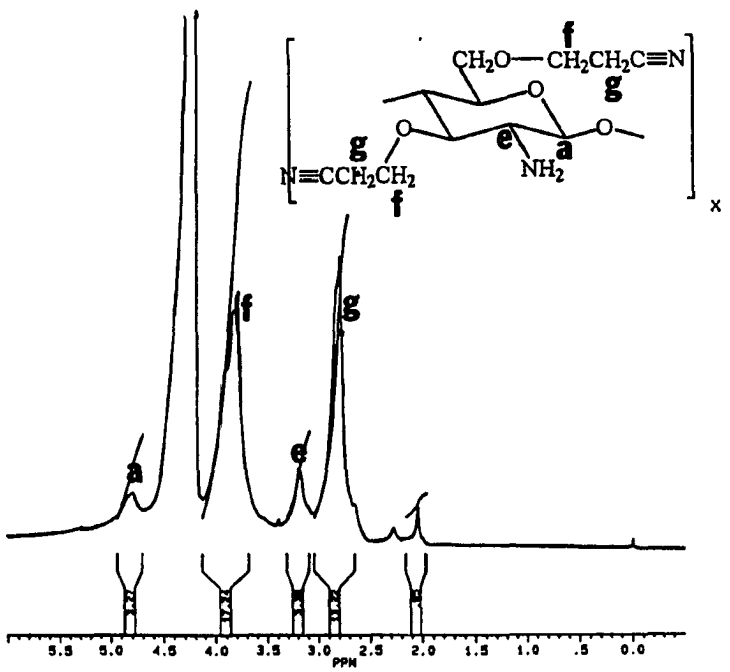


Figure 2.18 NMR of cyanoethyl chitosan.

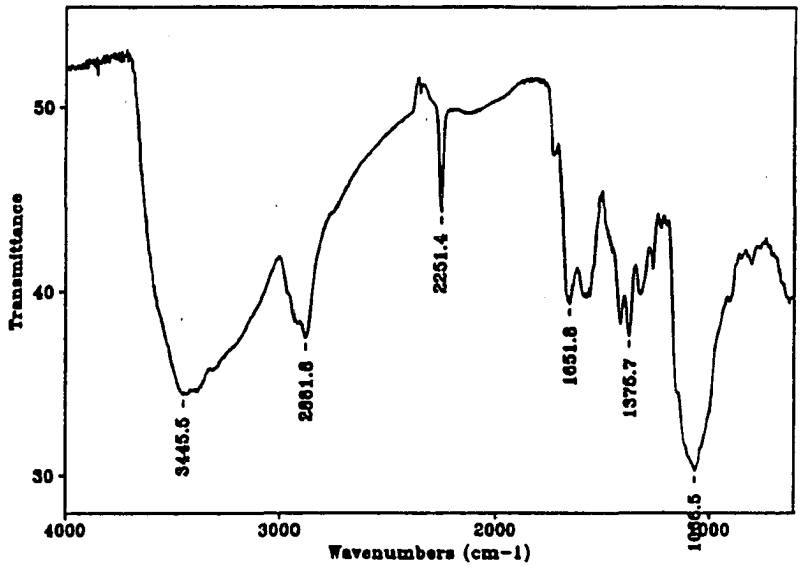


Figure 2.19 FTIR of R-cyanoethyl chitosan.

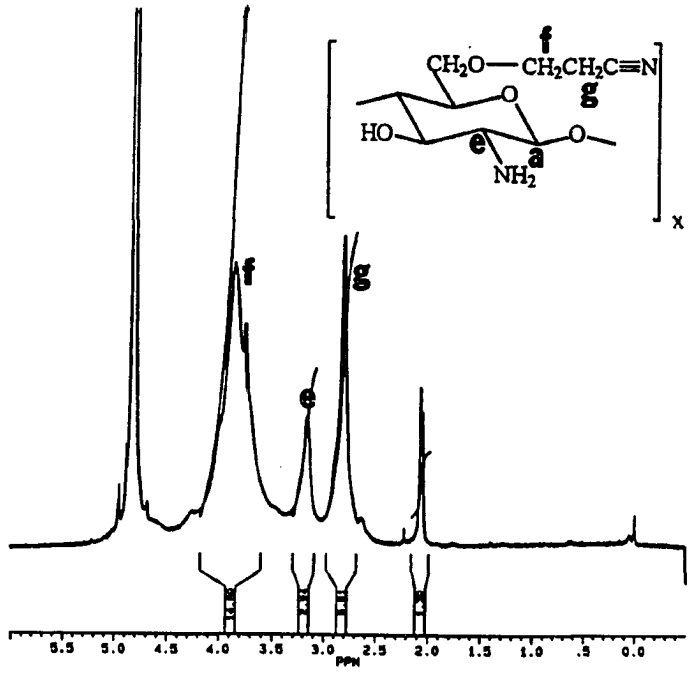


Figure 2.20 NMR of R-cyanoethyl chitosan.

Cyanoethyl chitosan was treated with $BH_3 \cdot S(CH_3)_2$ in tetrahydrofuran. For this reaction to proceed, dimethyl sulfide has to be removed by fractional distillation when the nitrile group reacts with borane forming a complex. However, when we attempted the reaction, as soon as the borane-dimethyl sulfide was added evidence of reaction (thanks to vigorous bubbling) was observed. Moreover, when distillation of dimethyl sulfide was attempted, all the solvent and reactant was passing through the column with no separation being achieved. We believe that complexation between borane and the primary amine, instead of complexation with the nitrile group, was responsible for this vigorous bubbling, thus explaining the failure of this reaction to reduce the nitrile group to a primary amine.

Attempts to reduce cyanoethyl chitosan with solid $LiAlH_4$ in diethyl ether and tetrahydrofuran for 72 hours were explored as well. As indicated by the IR (Figure 2.21) and NMR (Figure 2.22) spectra, some reduction occurred but the reaction did not take place quantitatively. The IR spectrum showed an almost complete disappearance of the nitrile peak, while the NMR spectrum, which indicates a D. S. = 0.9, shows that the signal for the methylene attached to the CN group (2.8 ppm)

decreased as well. The major disadvantage found in this reaction was the excessive amounts of $LiAlH_4$ ($LiAlH_4$/Cech 3.9/1) needed to reduce cyanoethyl chitosan. When the reducing agent is destroyed with water, aluminum hydroxide is formed, giving an insoluble contaminant and insoluble product with similar solubility properties, complicating the workup and purification of the product, which causes the recovery of less than 50% in weight of the starting material.

After R-Cech was obtained from regenerated chitosan, we tried to improve the reduction step by utilizing a solution of the reducing agent as well as a lower amount of it. A solution of $LiAlH_4$ in THF (which is sold commercially) was used trying to get a more swollen R-Cech during the reaction, but even though the ratio of $LiAlH_4$/CECH was 1.52/1, the starting material was not reduced after 48 hours at room temperature to any appreciable extent as proven by the IR spectrum, where the nitrile peak is still present at 2250 cm^{-1}.

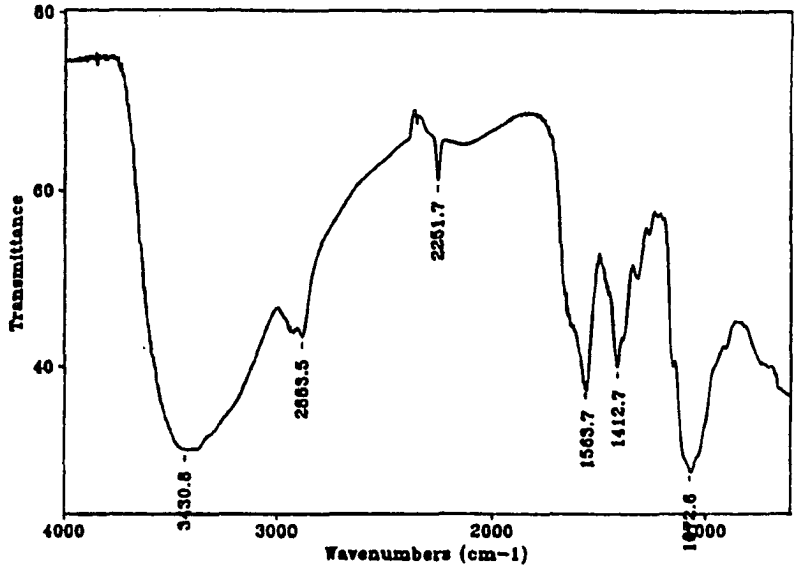


Figure 2.21 FTIR of aminopropyl chitosan.

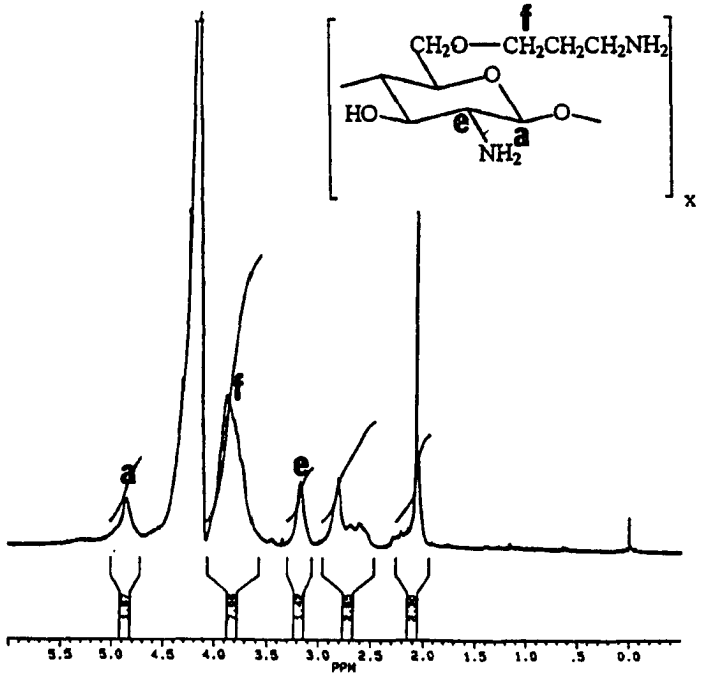
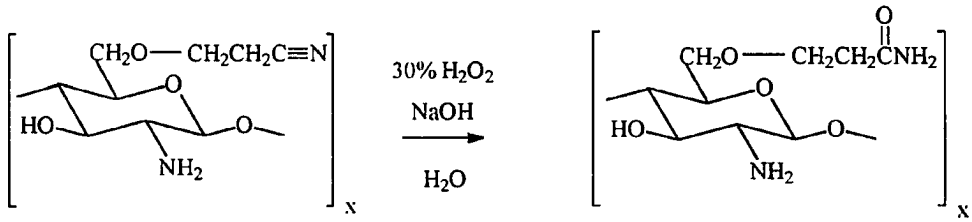


Figure 2.22 NMR of aminopropyl chitosan.

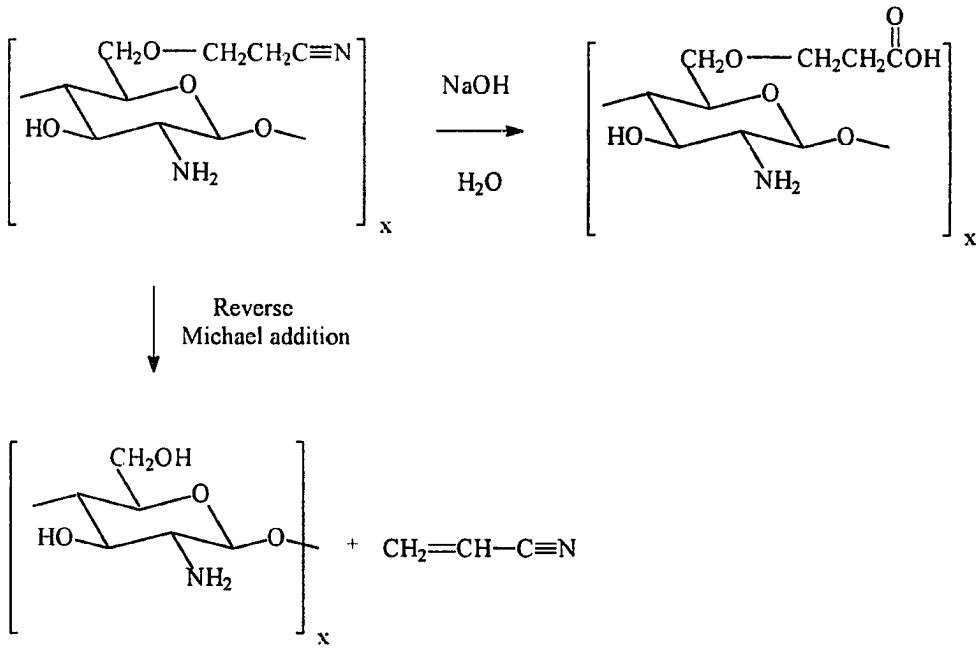
2.3.3.4 Regenerated carboxyamidoethyl chitosan (R-Caaech).



Scheme 2.10 Synthesis of carboxyamidoethyl chitosan.

In an effort to produce the amide derivative of R-Cech, the hydrolysis of the nitrile group to the amide was attempted using one of the mostly used methods to perform this reaction, which involves the use of H_2O_2 in NaOH.[117-121] The use of 30% H_2O_2 in a basic aqueous solution for 3 hours at 50°C permitted the hydrolysis of the nitrile group, which was confirmed by its disappearance in the IR spectrum (Figure 2.23); however, degradation of the polymer chain was evident by the drastic reduction in viscosity. Moreover, the solubility of the compound increased, but films could not be cast from solutions because the molecular weight of the derivative was too low. The NMR spectrum (Figure 2.24) indicates a D. S.= 1.4.

2.3.3.5 Regenerated carboxyethyl chitosan (R-Caech).



Scheme 2.11 Synthesis of carboxyethyl chitosan.

The hydrolysis of the nitrile group to the carboxylic acid moiety is one of the best methods available for the preparation of the latter.[117,122,123] However, when R-Cech was treated with NaOH in water at 60°C for 24 hours, the derivative obtained was not a water soluble product. The IR spectrum (Figure 2.25) does confirm the presence of a carboxylic acid group while the nitrile peak has disappeared completely, suggesting that the reaction took place in the desired fashion.On the other hand, the NMR spectrum (Figure 2.26), which indicates a D.S.=

0.2, did not show an acid peak; the signal of the methylene peaks attached to the nitrile group at 2.7 ppm did decrease as expected, but this spectrum resembled the chitosan NMR spectrum. We believe that a small amount of acid is present in this derivative, but that mostly a reverse Michael addition reaction has occurred during the attempted synthesis, going back to chitosan; thus explaining the insolubility of the product as well as its similarity in the NMR spectrum.

2.4 Deamination of chitosan.

We observed in the NMR spectrum that the cyanoethylation of chitosan had not occurred in position 2 (due to the fact that H-2 and H-1 did not move) as seen with the epoxides; rather, the spectrum suggested that reaction had actually taken place in position 6, where a small signal developed at around 4.3 ppm, which is consistent with the results reported by Nud'ga and coworkers,[113] and by Tokura et. al.[114] Moreover, it is reported that in cellulose, the alcohol in position 2 frequently reacts in etherifications, whereas in Michael addition reactions, the greatest extent of substitution occurs at the hydroxyl in position 6.[12] In order to confirm the results obtained by NMR,

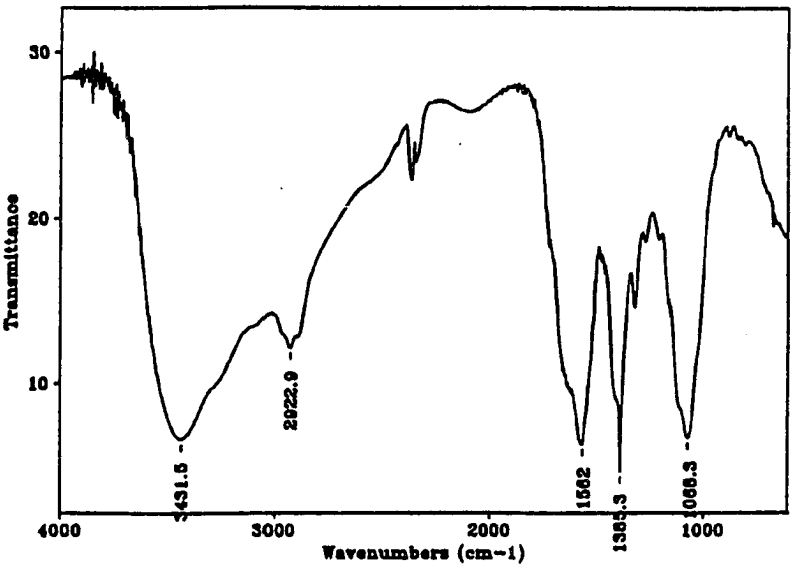


Figure 2.23 FTIR of R-carboxyamidoethyl chitosan.

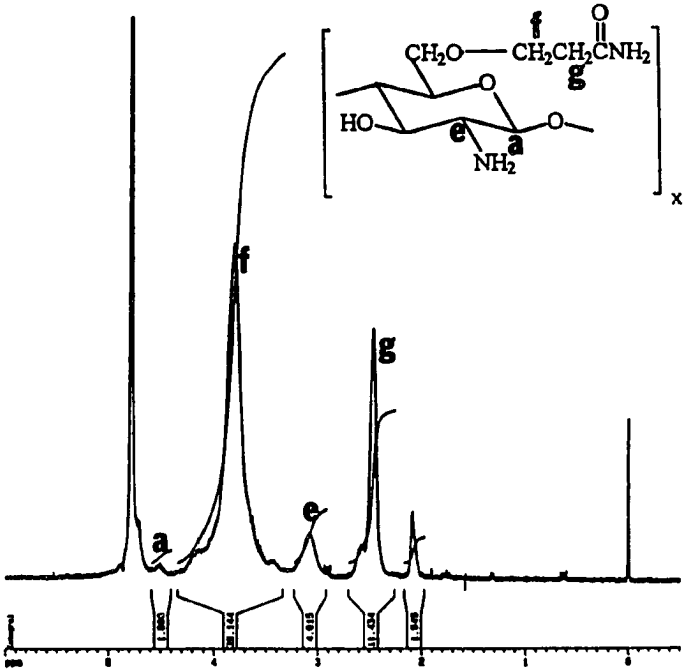


Figure 2.24 NMR of R-carboxyamidoethyl chitosan.

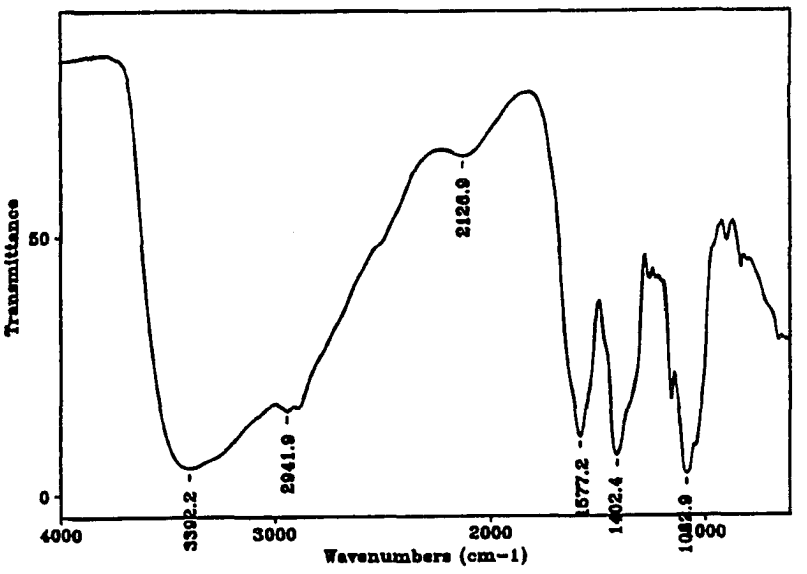


Figure 2.25 FTIR of R-carboxyethyl chitosan.

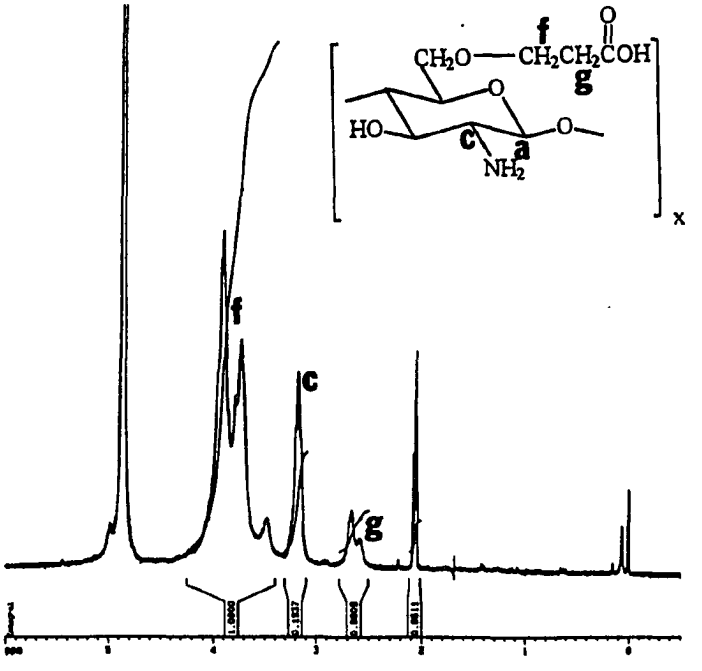


Figure 2.26 NMR of R-carboxyethyl chitosan.

Table 2.1 Solubility of oxirane-chitosan derivatives.

	dilute HOAc	dilute NaOH	H_2O	MeOH	EtOH	DMSO	Toluene	THF
Chitosa	+	-	-	-	-	-	-	-
Hech	+	+	+	-	-	+/-	+/-	-
R-Hech	+	+	+	+	+	+	+/-	-
Hpch	+	+	+	+	+	+	-	-
R-Hpch	+	+	+	+	+	+	-	-
Hbch	+	-	-	-	-	+/-	-	-
Hphch	-*	-	-	-	-	-	-	-
R-ch-quat18	+	+	+	+/-	+/-	+/-	+/-	-

* only soluble in 15% acetic acid.

+ soluble.

- insoluble.

+/- partially soluble or swollen.

Table 2.2 Solubility of acrylonitrile-chitosan derivatives.

	dilute HOAc	dilute NaOH	H_2O	MeOH	EtOH	DMSO	Toluene	THF
Chitosan	+	-	-	-	-	-	-	-
Cech	+	-	-	-	-	-	-	-
R-Cech	+	+/-	+/-	-	-	+/-	+/-	-
Apch	+	-	+	-	-	-	-	-
R-Caaech	+	+	+	-	-	+/-	+/-	-
R-Caech	+	+/-	+/-	-	-	+/-	+/-	-

+ soluble.

- insoluble.

+/- partially soluble or swollen.

Table 2.3 Yields of oxirane-chitosan derivatives.

starting compound	grams used	mol equiv. used	product	grams obtained	D. S.	M. S.
Chitosan	1	0.006	Hech	2.97	1.4	11.1
Chitosan	1	0.006	R-Hech	1.32	1.5	4.4
Chitosan	1	0.006	Hpch	1.84	1	4
Chitosan	1	0.006	R-Hpch	1.60	2.2	5.6
Chitosan	1	0.006	Hbch	0.96	0.1	
Chitosan	1	0.006	Hphch	1.50	0.5	
Chitosan	1	0.006	R-ch-quat188	1.27	1.1	0.9

Table 2.4 Yields of acrylonitrile-chitosan derivatives.

starting compound	grams used	mol equiv used	product	grams obtained	D. S.	M. S.
Chitosan	4	0.023	Cech	5.97	2	
Chitosan	1	0.006	R-Cech	0.99	1	
Cech	3		Apch	1.15	0.9	
R-Cech	1		R-Caaech	0.90	1.4	
R-Cech	1		R-Caech	0.48	0.2	

Mol. Wt. equiv. = (x)(Mol. Wt. chitosan) + (y)(Mol. Wt. chitin)

x = mol fraction of chitosan = 0.79; Mol. Wt. chitosan = 161 g mol

y = mol fraction of chitin = 0.21; Mol. Wt. chitin = 203 g mol

mol equivalent = $\frac{\text{(grams of chitosan)}}{\text{(Mol. Wt. equivalent)}}$

we depolymerized chitosan by reacting it with nitrous acid and analyzed the resulting product.

Amines are known to react with nitrous acid (which is prepared in situ from sodium nitrite and a strong acid) to yield different compounds depending upon the amine in question. Primary amines (aliphatic or aromatic) react with nitrous acid to produce a diazonium salt, which might be stable or not depending on the substituents. If the salt is not stable, it will lose nitrogen producing a carbocation intermediate, which presents the potential for rearrangements.

$$R\text{-}NH_2 + NaNO_2 + HCl \rightarrow R\text{-}N_2^+ Cl^- \rightarrow R^+ Cl^- + N_2$$

Secondary amines on the other hand (both aliphatic and aromatic), when treated with nitrous acid, produce relatively stable products called N-nitrosoamines.

$$R_2\text{-}NH + NaNO_2 + HCl \rightarrow R_2\text{-}N\text{-}NO$$

If nitrous acid is reacted with a tertiary aliphatic amine, the products obtained will be the amine salt and the N-nitrosoammonium derivative.

$$R_3\text{-}N + NaNO_2 + HCl \rightarrow R_3\text{-}N^+H\ Cl^- + R_3\text{-}N^+\text{-}NO\ Cl^-$$

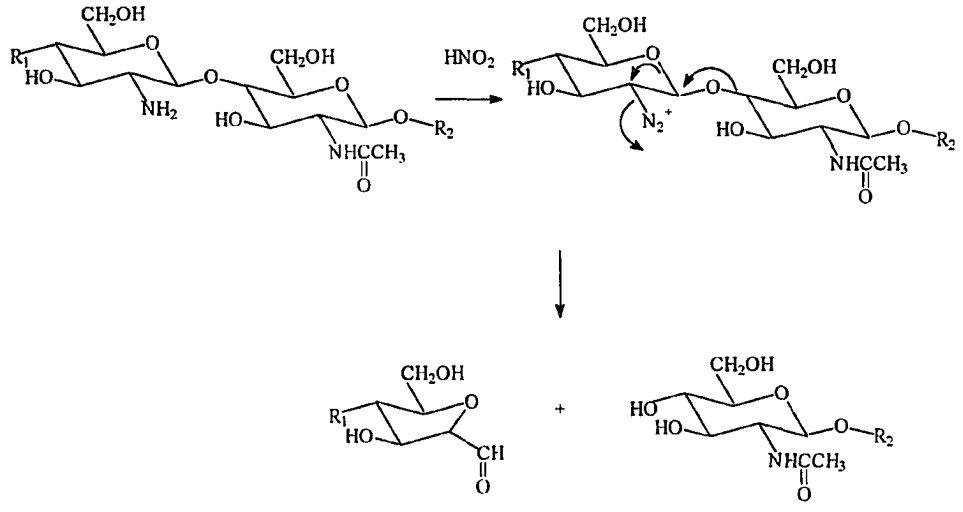
When polysaccharides and glycoproteins containing 2-amino-2-deoxy-D-glucopyranose units are treated with nitrous acid, a controlled

degradation that yields 2,5-anhydro-D-mannose occurs.[33,124-126] However, the use of nitrous acid to deaminate chitosan is not the only method reported in the literature. Treatments of the polysaccharide with barium hypobromite, silver nitrate in hydrochloric acid solution, and reaction with gaseous dinitrogen trioxide have been also reported to promote the depolymerization of chitosan.[93]

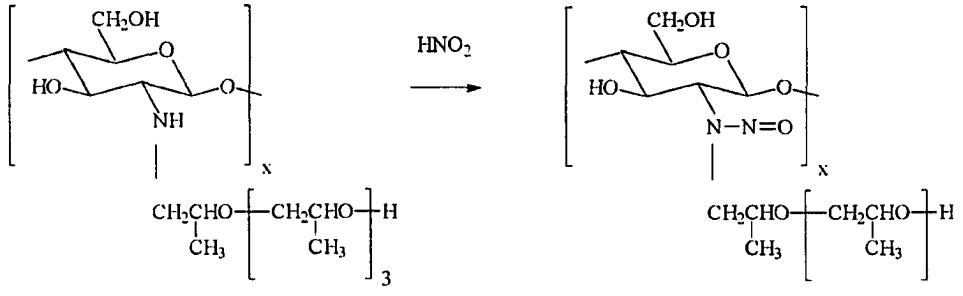
When chitosan is deaminated, the portion bearing the primary amine will form a diazonium salt, which then loses nitrogen and forms 2,5-anhydro-D-mannose as evidenced by the decrease in viscosity. However, the portion bearing the N-acetyl group (an amide) will not undergo deamination. The latter fact explains why we still see H-2 peaks at 3.2 ppm in the NMR spectrum in the oligomeric residues (Figure 2.27).

When hydroxypropyl chitosan (D. S. = 1) was characterized, the NMR spectrum revealed that reaction took place at the nitrogen in position 2 (confirmed by the upfield shift of H-2 and H-1). This secondary amine was then treated with nitrous acid, rendering the N-nitrosoamine intermediate, which caused a downfield shifting (from the region 2.5-3.0 ppm) to H-2 and the two hydrogens from the CH_2 directly

attached to the nitrogen, and a downfield shift to H-1 from 4.5 ppm to 5.05 ppm (Figure 2.28).



Scheme 2.12 Deamination of chitosan.



Scheme 2.13 Nitrosation of hydroxypropyl chitosan.

If chitosan reacted with acrylonitrile to produce cyanoethyl chitosan with the substituent at position 2, the product obtained would be a secondary amine, which should cause H-2 and H-1 to move upfield

(not observed). This derivative should react with nitrous acid similarly to hydroxypropyl chitosan, forming the N-nitrosoamine intermediate, which should move H-2 and H-1 downfield (not observed).

However, if chitosan reacted with acrylonitrile to produce cyanoethyl chitosan with the substituent at position 6, no upfield movements should occur to H-2 and H-1 (consistently with data data obtained). The product obtained could contain a primary amine as well as an amide functionality due to the chitosan backbone. Therefore, this derivative should deaminate where primary amine groups exist, but it should not where amide groups exist, leaving the residual H-2 and H-1 unchanged (consistently with data obtained in Figure 2.29). Furthermore, when the integral of H-2 is compared to the integral of the carbohydrate ring, its ratio decreases from 20% (before deamination) to 5% (after deamination), which indicates that the signal observed for H-2 should be only from residual N-acetyl groups found in chitosan. Therefore, we can conclude that the cyanoethylation of chitosan occurred at position 6 rather than 2, and the deamination is following the pathway shown in Scheme 2.15.

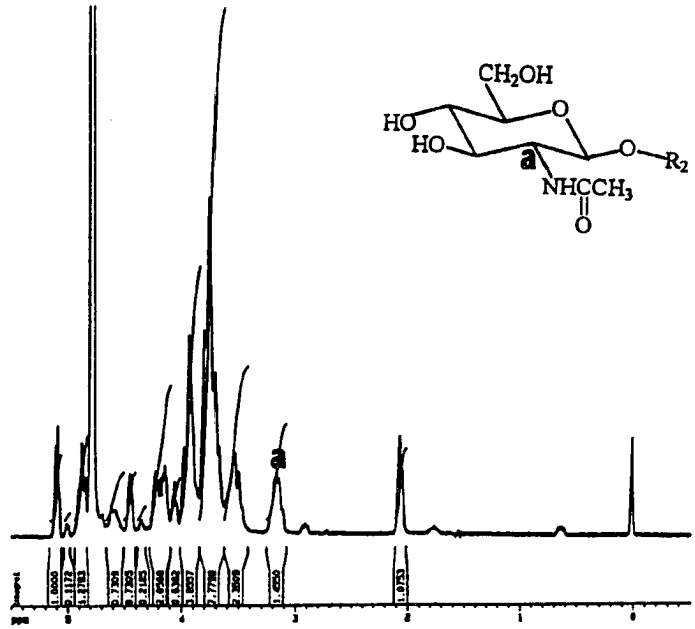


Figure 2.27 NMR of deaminated chitosan.

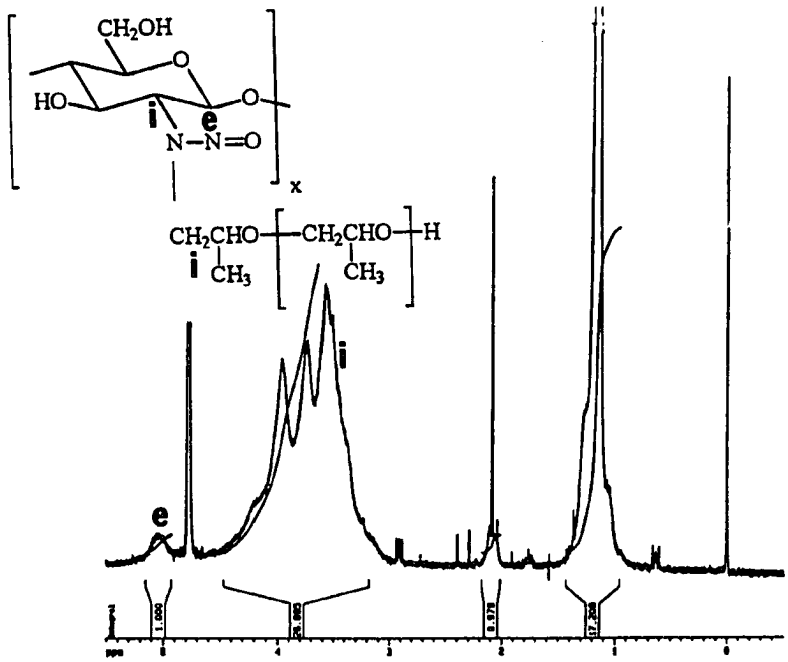
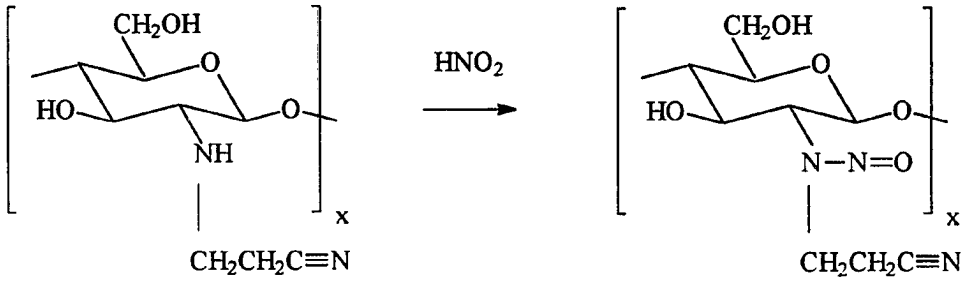
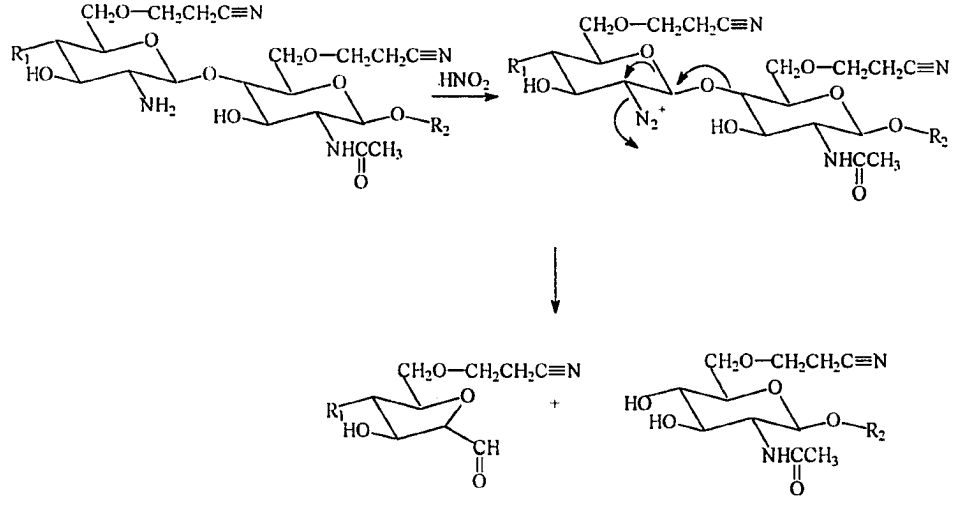


Figure 2.28 NMR of deaminated hydroxypropyl chitosan.



Scheme 2.14 Nitrosation of N-cyanoethyl chitosan.



Scheme 2.15 Deamination of O-cyanoethyl chitosan.

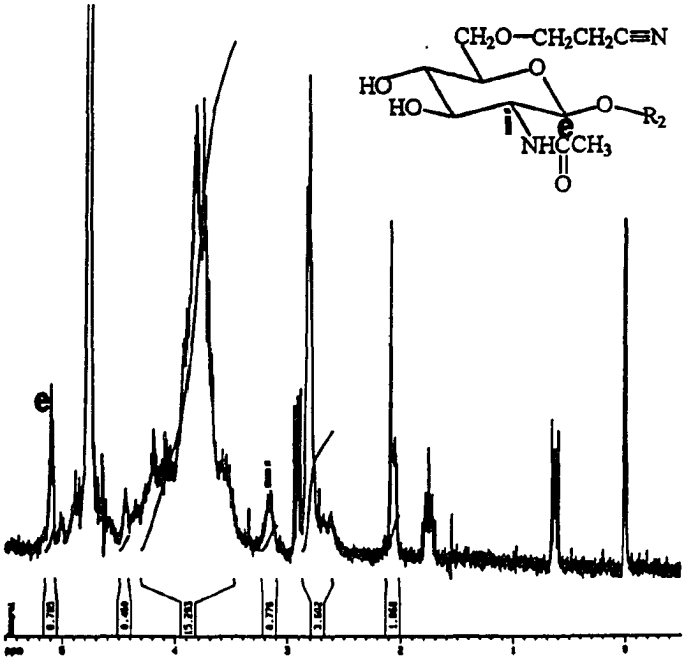


Figure 2.29 NMR of deaminated R-cyanoethyl chitosan.

CHAPTER THREE

PHYSICAL CHARACTERIZATION OF CHITOSAN AND ITS DERIVATIVES

3.1 Properties of chitosan.

Chitin is insoluble in most common solvents, but its dissolution in DMAc-LiCl solutions is possible. On the other hand, chitosan is insoluble in H_2O, bases and in common organic solvents, such as DMSO, DMF, NMP and alcohols. However, it dissolves in aqueous solutions of organic acids like acetic, formic, citric, glycolic, lactic and others.[127]

Since the pKa for the amino groups present in chitosan is between 6.0 and 7.0,[128-130] they can be protonated in very dilute acids close to neutral conditions, rendering a cationic nature to this biopolymer, which is the basis of many of the potential applications of chitosan.[73] Chitosan can be considered as a linear polyelectrolyte with a high charge density which can interact with negatively charged surfaces, like proteins and anionic polysaccharides. Thus, it acts as a good flocculant, and its ability to adhere to natural polymers such as hair and skin make it valuable as a component of cosmetics and shampoos.

3.2 Characterization.

The determination of the molecular weight of a polymer is one of the most important aspects in polymer chemistry. Without the knowledge of this parameter, no real understanding between structure and properties can be made. The use of chemical techniques for determining the molecular weight of macromolecules, such as end group analysis, is limited due to the fact that these methods are rather insensitive for polymers with large molecular weights (over 25,000).[131] However, physical methods provide us with more reliable alternatives. Two different approaches (absolute and secondary methods) are used to determine molecular weight of polymers.

Absolute methods (osmotic pressure, cryoscopy, ebulliometry, light scattering and ultracentrifuge techniques) provide a direct determination of the molecular weight. Secondary methods (solution viscosity and gel permeation chromatography), which have to be calibrated utilizing absolute techniques, give an indirect determination of the molecular weight.

Determination of the molecular weight of chitosan has been achieved by viscometry,[132-134] gel permeation chromatography,[22,28]

sedimentation,[134] osmometry,[27] and light scattering[27,133-137]. Wang et. al.[133] determined the k and α values in the Mark-Houwink equation for chitosan samples with degrees of deacetylation from 69% to 100%. They observed that α decreased, indicating that the rigidity of the chains decreased, as the degree of deacetylation increased, which was attributed to the fact that less hydrogen bonding was present in the biopolymer. In contrast, k increased as the degree of deacetylation increased; this effect was explained in terms of the polyelectrolyte effect, where the electrostatic repulsion of the ionic groups along the chitosan chain promote the expansion of the chitosan coil as the degree of deacetylation increases, causing the intrinsic viscosity to rise.

Beri and coworkers[136] characterized several chitosan samples using size exclusion chromatography-multiangle laser light scattering (SEC-MALLS), obtaining results that showed reproducibility in molecular weight and radius of gyration data. These data was compared with static light scattering determinations, showing good agreement between both techniques and with values reported in the literature. Furthermore, they compared the data from SEC-MALLS with data from SEC using dextran standards as calibrants (to compare molecular

weights reported by other researchers). It was found that the molecular weights using SEC were 2-3 times higher than those obtained from SEC-MALLS. The discrepancy was a difference in conformation between the randomly coiled dextran used as the calibrant and the extended worm-like chain conformation of chitosan in solution.

In 1995, Wu and collaborators[137] established a relationship between the molecular weight and the translational diffusion coefficient. They observed in this study that the chitosan chain (91% deacetylated) is slightly extended in aqueous solution even in the presence of salts due to its backbone and polyelectrolyte nature.

3.2.1 Viscosity determinations.

3.2.1.1 Intrinsic viscosities.

Even though viscometry is a secondary method, it is one of the simplest and most rapid techniques available to determine the molecular weight of a polymer sample. This method is based on the Mark-Houwink equation:

$$[\eta] = k \times M^{\alpha}$$

where [η] is the intrinsic viscosity (viscosity extrapolated to zero concentration), M is the molecular weight, and k and α are constants determined experimentally for polymer-solvent systems.

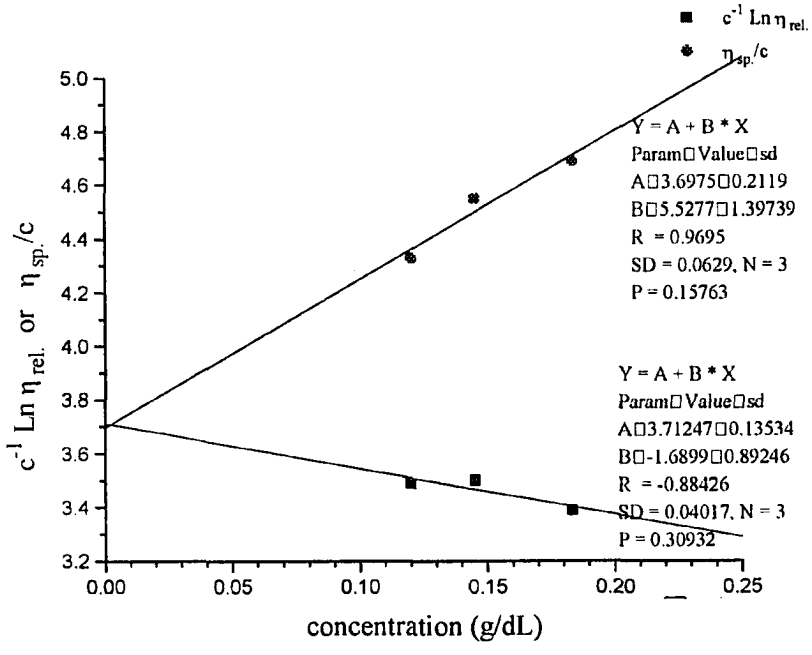
In order to obtain the viscosity average molecular weight of chitosan, it is necessary to have a good solvent as well as the k and α parameters for the Mark-Houwink equation. Several solvent systems have been proposed in the past for the determination of these parameters,[132,133] but aggregation factors and the degree of deacetylation were not taken into account in these studies. Nowadays, the solvent system mostly used is a buffer solution of acetic acid/sodium acetate,[133-137] which has been utilized to prepare solutions avoiding aggregation.[135] In 1991, Wang and coworkers determined the k and α parameters for the Mark-Houwink equation for several chitosan samples with different degrees of deacetylation using 0.2M acetic acid/0.1M sodium acetate as the solvent system.[133] The authors developed equations for determining k and α as a function of the degree of deacetylation:

$$k = (1.64 \times 10^{-30}) \times (DD^{14.0}) \ (mL/g)$$

$$\alpha = (-1.02 \times 10^{-2}) \times (DD) + 1.82$$

where DD is the % degree of deacetylation.

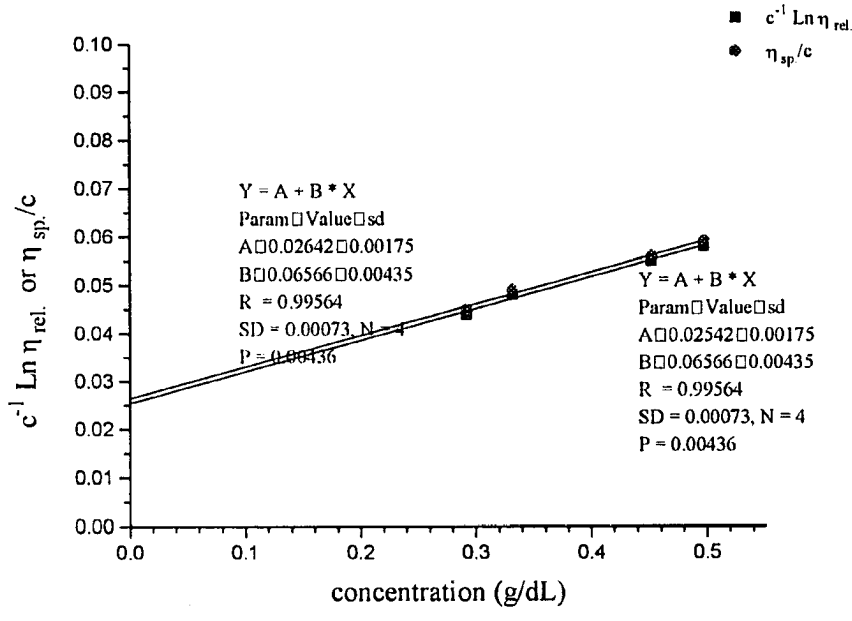
The degree of deacetylation calculated by FTIR in our sample was found to be 79 %, giving a k = 0.6048 × 10^{-3} mL/g and α = 1.0142. The intrinsic viscosity for chitosan was determined to be 370.5 $\pm$ 0.7 mL/g. Thus, the molecular weight of the biopolymer was calculated using the Mark-Houwink equation to be a viscosity average molecular weight = 552,000 daltons.



Plot 3.1 Intrinsic viscosity of chitosan.

We proceeded to determine the intrinsic viscosities for all the chitosan derivatives prepared (Table 3.1). However, due to the lack of the k and α parameters, we did not calculate their molecular weight.

Nevertheless, it is clear that some degradation occurs during the modification process. As observed in Plot 3.2, carboxyamidoethyl chitosan shows very low viscosities for all the concentrations, and therefore, a very low intrinsic viscosity because its chain was extensively degraded during the reaction, where peroxides were used.



Plot 3.2 Intrinsic viscosity of regenerated carboxyamidoethyl chitosan.

3.2.1.2 Cone and plate viscosities.

In order to determine the behavior shown by the solutions of chitosan and its derivatives under the presence of external forces, determinations of their viscosities using a cone and plate instrument were

Table 3.1 Intrinsic viscosities of chitosan and its derivatives.

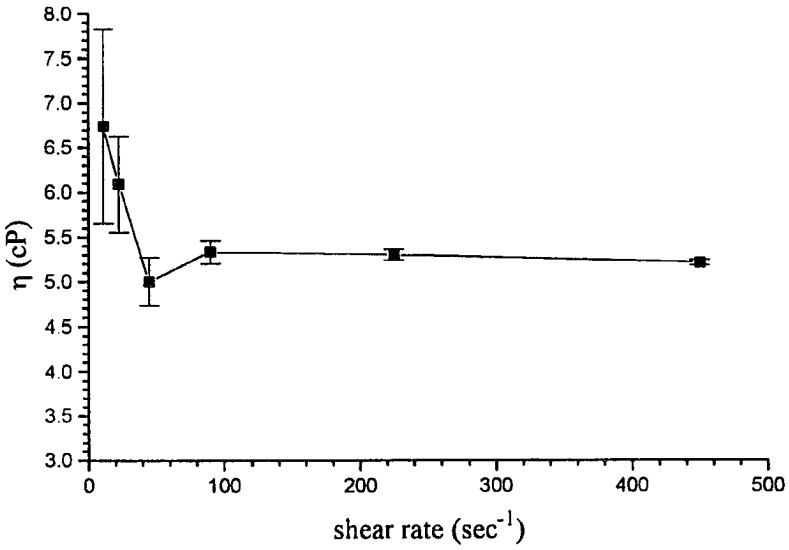
Compound	[η] (dL/g)
Chitosan	3.705 ± 0.007
Hydroxyethyl chitosan	1.105 ± 0.019
R-Hydroxyethyl chitosan	1.139 ± 0.003
Hydroxypropyl chitosan	1.325 ± 0.025
R-Hydroxypropyl chitosan	2.137 ± 0.009
Hydroxybutyl chitosan	1.142 ± 0.002
R-Chitosan-quat 188	1.354 ± 0.004
R-Cyanoethyl chitosan	1.841 ± 0.007
R-Carboxyamidoethyl chitosan	0.0255 ± 0.0005

performed. When an external force is applied to a solution, its viscosity will vary with the rate of deformation, presenting a Newtonian (viscosity is independent of shear rate), shear thickening (viscosity increases with shear rate), shear thinning (viscosity decreases with shear rate) or a Bingham plastic behavior.[138,139] Shear thickening effects are best exemplified by particulate dispersions, such as latexes, slurries, and concentrated suspensions; but if slurries and concentrated suspensions

behave like solids (no flow) until the shear stress exceeds a certain value (yield stress), after which they flow readily, the sample shows a Bingham plastic behavior (i. e. toothpaste). Most polymer solutions, as well as polymer melts, typically show a shear thinning behavior.

Polysaccharides and their derivatives exhibit different rheological behaviors, ranging from Newtonian to various types of non-Newtonian flow properties.[140] Hydroxyethyl cellulose and hydroxypropyl cellulose, similar to other cellulose ethers, show Newtonian behavior at low shear rates, but become pseudoplastic (shear thinning) at higher shear rates; the shear rate at which solutions change from Newtonian to shear thinning increases with higher molecular weight and lower concentrations.[141]

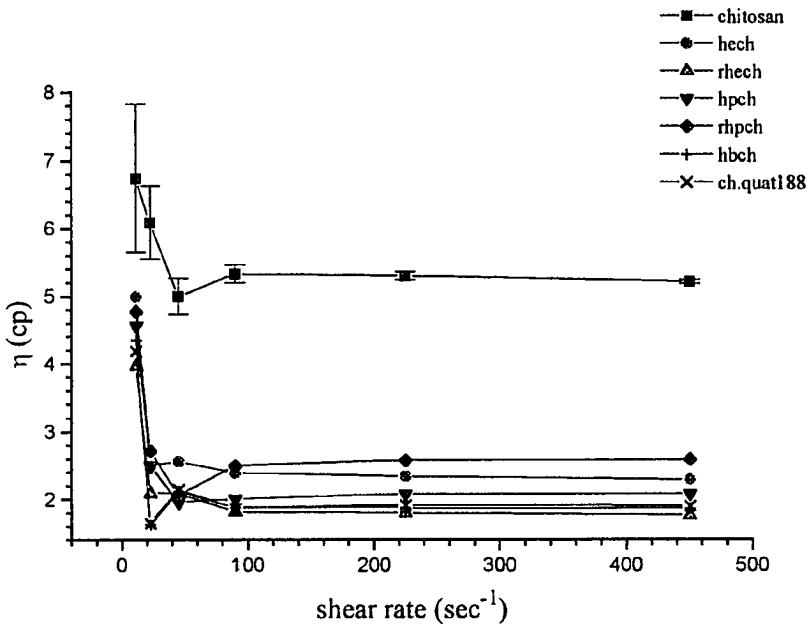
The behavior observed for chitosan as well as for its oxirane derivatives may be shear thinning, but the large error associated with the measurements at low shear rate makes it difficult to assess this property (Plot 3.3 and 3.4). Chitosan presents higher viscosities than any of the derivatives synthesized due to the strong intra- and inter-molecular hydrogen bonding present in the polysaccharide and due to the chain expansion promoted by the positively charged ammonium groups.



Plot 3.3 Cone and plate viscosity of chitosan at 0.5%.

It is observed in Plot 3.4 that hydroxyethyl chitosan (M. S. = 11.1) and regenerated hydroxypropyl chitosan (M. S. = 5.6) show higher viscosities than their analogues (regenerated hydroxyethyl chitosan M. S. = 4.4 and hydroxypropyl chitosan M. S. = 4), suggesting that for a given derivative, the viscosity will increase with a higher M. S. regardless of the method used to synthesize it.

Similarly to the behavior observed for chitosan and its oxirane derivatives, chitosan and its acrylonitrile derivatives also present a behavior which resembles shear thinning (Plot 3.5). The derivatization of

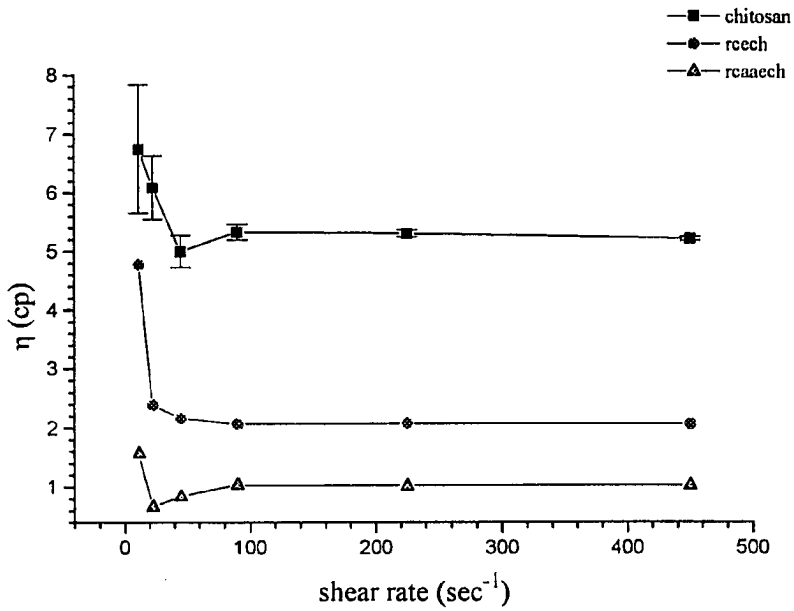


Plot 3.4 Cone and plate viscosities of chitosan and chitosan-oxirane derivatives at 0.5%.

chitosan to produce regenerated cyanoethyl chitosan resulted in the same effect observed in the oxirane derivatives, lower viscosity values. Moreover, when regenerated cyanoethyl chitosan was reacted to produce regenerated carboxyamidoethyl chitosan, the viscosity dropped to 1 cP due to the use of hydrogen peroxide in the reaction, which cleaved the polymeric chain, and lowered the viscosity drastically.

3.2.2 Thermal Analysis.

The knowledge of the thermal properties of a polymeric substance is always important and often critical in both processing stages and



Plot 3.5 Cone and plate viscosities of chitosan and chitosan-acrylonitrile derivatives at 0.5%.

product uses. In order to understand and learn the transitions occurring in polymers when subjected to temperature changes, these substances are studied by thermal analysis, which is the tool that provides us with information such as decomposition temperatures, melting temperatures, and glass transition temperatures. The techniques mostly used when thermal analysis is performed are thermogravimetry (TG), which gives gain or loss of weight (mass change); differential thermal analysis (DTA) and differential scanning calorimetry (DSC), which give change in specific heat capacity (rate of enthalpy change). Although the techniques

already mentioned are the most common, several others are becoming widely used, i. e., thermomechanical analysis (TMA) and thermodilatometry, give penetration or expansion (dimension change); dynamic mechanical analysis (DMA) and dielectric thermal analysis (DETA), give loss moduli; evolved gas detection (EGD) and evolved gas analysis (EGA), among others.[142]

There have been some reports in the literature regarding the study of thermal properties of chitosan.[143-148] In these articles,[144-148] it has been found in the TG and DSC thermograms that after a decrease in the weight due to water loss, chitosan has only one main degradation step at around 300°C. This effect can be explained in terms of the rigidity of the polymeric backbone in polysaccharides, where observation of the glass transition and melting temperatures is hampered by thermal degradation.[145,148]

Consistent with the literature reports, we were able to detect only the loss of water and the degradation step for chitosan and its derivatives. It is observed in the TG thermograms (Figure 3.1 and 3.2) that the temperature where decomposition starts (onset temperature) is lower (40 to 50°C) for all the derivatives obtained than for chitosan. This

effect can be attributed to the incorporation of different groups to the biopolymer, which have disrupted the strong intra- and inter-molecular hydrogen bonding responsible for the rigidity of chitosan.

Table 3.2 Thermogravimetric analysis of chitosan and its derivatives.

Compound	Onset temp.	% wt. loss	Final temp.	% wt. loss
Chitosan	242.8°C	10	332.3°C	47.7
R-Cech	203.6°C	12.2	352.4°C	67.9
R-Caaech	156.7°C	5.2	350.4°C	26.9
Hech	194.6°C	7.8	366.9°C	77.4
R-Hech	191.3°C	4.5	365.8°C	73.7
Hpch	175.6°C	7.1	347.9°C	79.6
R-Hpch	195.8°C	10.4	347.9°C	81.1
Hbch	200.2°C	17.6	340.1°C	71.3
Hphch	200.3°C	12.6	318.1°C	59.2
R-Ch-quat 188	190.2°C	18.7	290.9°C	60.7

Furthermore, some degradation of chitosan occurred during the synthesis of the derivatives (confirmed by a decrease in viscosity), producing a

decrease in the onset temperature, as exemplified by R-Caaech (Figure 3.3), which was severely degraded during its synthesis and has a much lower onset temperature than chitosan or any other derivative obtained. As expected, the % weight loss at the end of decomposition for all the derivatives (except R-Caaech), where we first lose the side chain and then degrade the main polymeric chain, is higher than the % weight loss for chitosan, where we only degrade the main chain.

It was observed in Plot 3.4 that hydroxyethyl chitosan (M. S. = 11.1) and regenerated hydroxypropyl chitosan (M. S. = 5.6) had higher viscosities than their analogues (regenerated hydroxyethyl chitosan M. S. = 4.4 and hydroxypropyl chitosan M. S. = 4). A similar behavior is observed in the thermogravimetric analysis (Figure 3.4), where hydroxyethyl chitosan and regenerated hydroxypropyl chitosan start decomposing at higher temperatures; the % weight loss at the end of decomposition is higher for them than for their analogues (regenerated hydroxyethyl chitosan and hydroxypropyl chitosan), confirming the fact that these properties depend on the M. S. and not on the synthetic method utilized to obtain them.

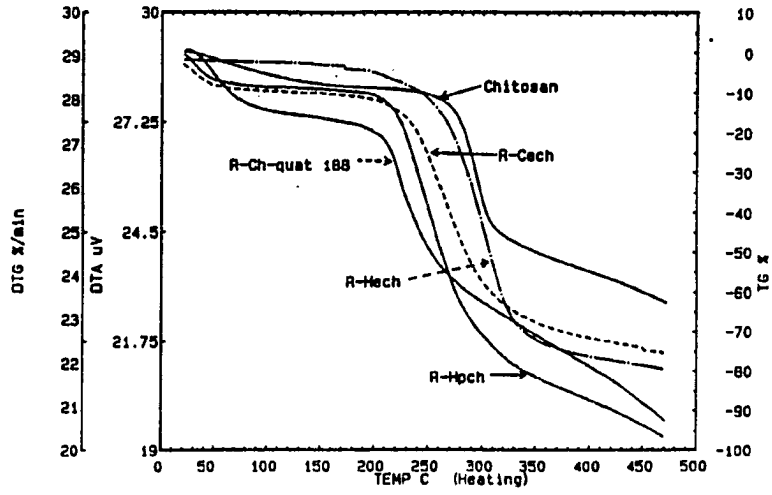


Figure 3.1 TG thermogram of chitosan and the regenerated derivatives.

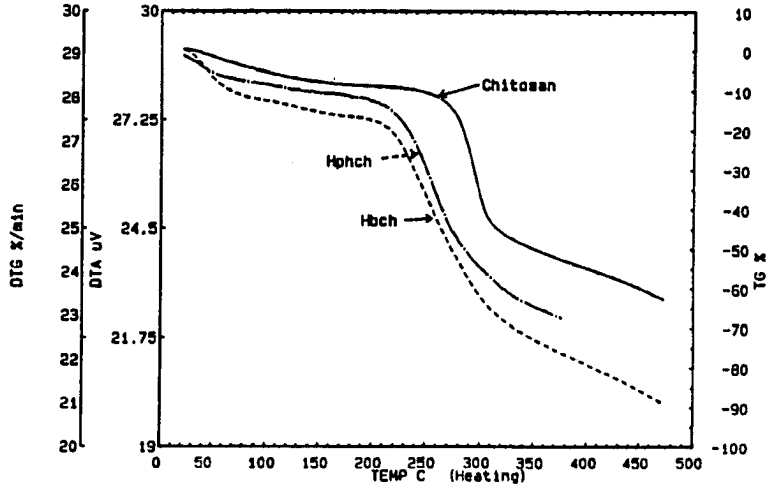


Figure 3.2 TG thermogram of chitosan, hydroxybutyl chitosan, and hydroxy(2-phenyl)ethyl chitosan.

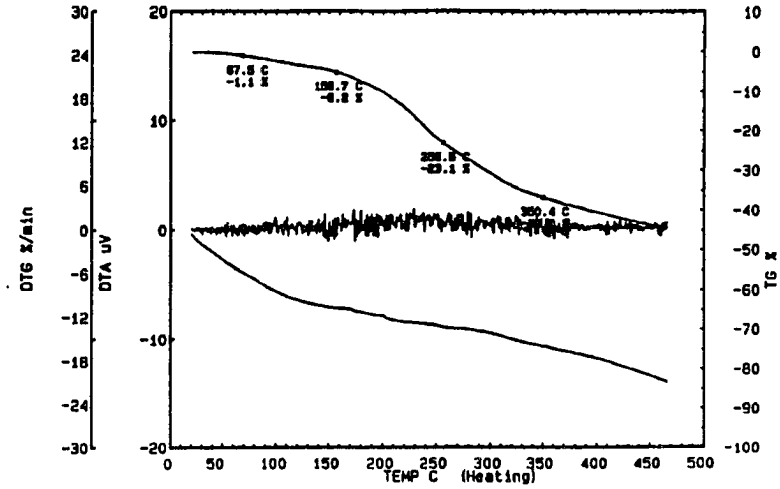


Figure 3.3 TG thermogram of R-carboxyamidoethyl chitosan.

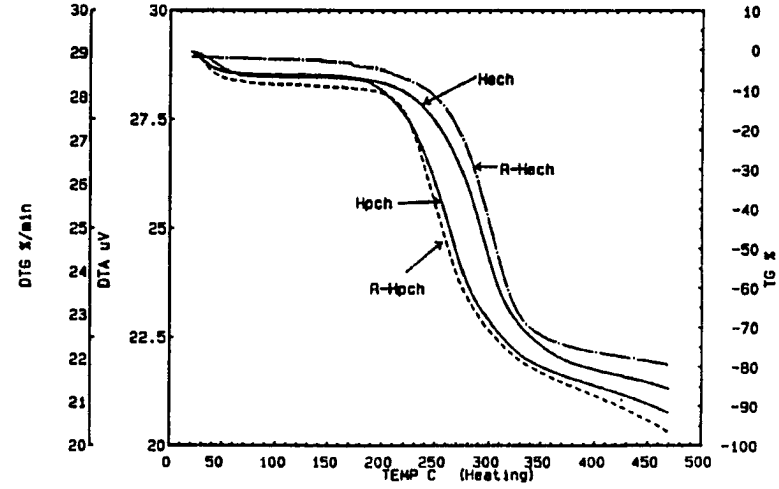


Figure 3.4 TG thermogram of hydroxyethyl chitosan, hydroxypropyl chitosan, and R-analogues.

A common feature in the DSC thermograms for chitosan and its derivatives is a large endotherm due to water evaporation, which depending on the compound, is found between 30°C-130°C (Figure 3.5). The variable location of this endotherm is consistent with the literature.[144-148] Hydroxyethyl chitosan (M. S. = 11.1) shows a glass transition temperature at -54.1°C, while regenerated hydroxyethyl chitosan (M. S. = 4.4) presents two peaks at -51.3°C and -41.3°C rather than a well defined glass transition temperature (Figure 3.6).

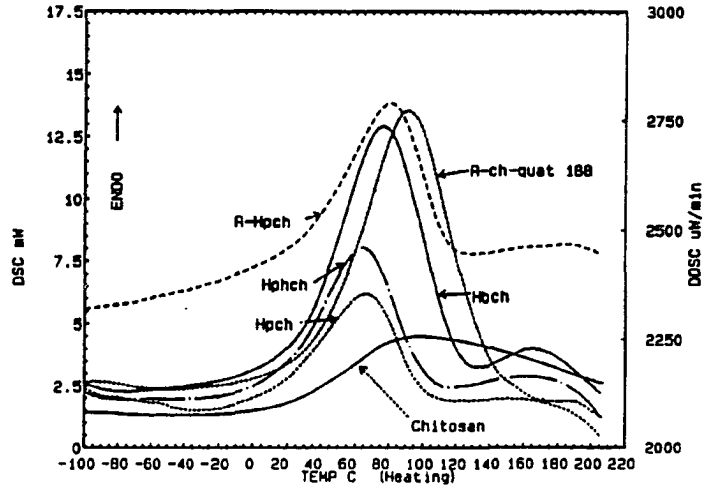


Figure 3.5 DSC thermogram of chitosan and its derivatives.

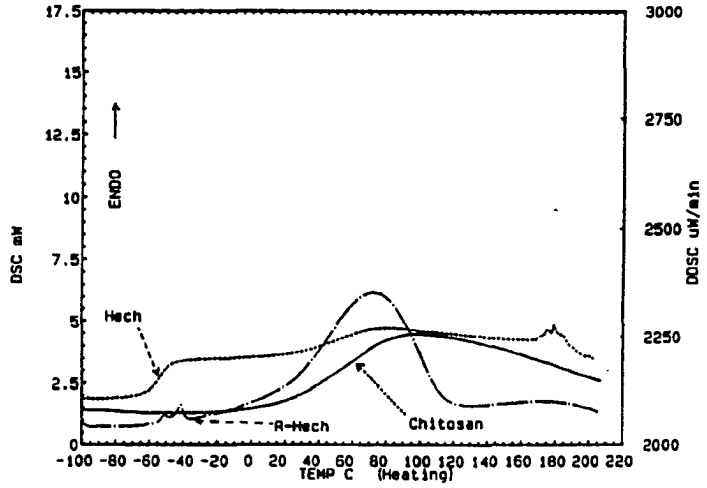


Figure 3.6 DSC thermogram of hydroxyethyl chitosan, and R-hydroxyethyl chitosan.

CHAPTER FOUR

FLOCCULATION ACTIVITY

4.1 Flocculating agents.

When small suspended particles or colloids do not permit the separation of solids and liquids, due to the presence of an electrically charged atmosphere surrounding each particle, which generates repulsive forces between particles approaching each other, the result is long sedimentation or filtration times.[149,150] In order to alleviate this problem, flocculating or coagulating agents are added to the suspension, which will increase the particle size (forming flocs) and facilitate the rapid separation or drainage of the liquid phase from the solid phase. The main applications of flocculating agents in the industry are in raw and waste-water clarification, sludge dewatering, mineral processing and papermaking.[149-152]

4.1.1 Mechanism of action of flocculating agents.

4.1.1.1 Charge neutralization.

The stability of aqueous suspensions of small particles is due mainly to two factors. First, Brownian motion is responsible for preventing particle settling, while electrostatic repulsion due to surface

charges of the particles, which are arranged in an electrical double layer, prevents collision and aggregation. This electrical double layer is composed of an initial layer of adsorbed ions and molecules located at the particle surface called the Stern layer, which presents a charge to the solution attracting a diffuse layer of free ions with an opposite charge, known as the Gouy-Chapman layer.[149] Neutralization of this charge can be accomplished by compressing the double layer through an increase in solution ionic strength (addition of sodium chloride), permitting the colloidal particles to approach each other, resulting in flocculation of stable suspensions. Another approach often employed to effect charge neutralization is to use hydrolyzable aluminum and iron salts, where the charge is neutralized by adsorption of these species onto the colloidal particle surface. Polymers flocculate suspended particles by a combination of two mechanisms, bridging and charge neutralization.

4.1.1.2 Bridging.

Bridging is the flocculation mechanism where polymeric molecules act as links between small particles. This effect occurs when relatively few segments of a polymer attach onto the colloid particle surface while the unattached segments extend into the bulk of the

solution. This configuration provides for an adsorbed polymer layer which, because of the loops and trains formed by this random attachment of segments, spans the distance of closest approach to other particles. This increases the particle collision-diameter and provides a point of attachment beyond electrostatic repulsive forces, thus promoting flocculation to occur. Sufficiently long polymer extensions from the points of attachment as well as vacant adsorption sites at the point of collision are necessary for bridging to occur.

4.2 Types of flocculants.

The inorganic flocculants more frequently used in the industry are based upon the hydrolyzable salts aluminum sulfate and ferric chloride. However, the use of polymeric flocculants in most industrial applications today has replaced almost completely the use of these salts. The reason for this fact lies on the advantages presented by the polymeric flocculants, i. e., they produce larger, stronger and more rapidly formed flocs, there is no need to add salt to the system, and less sludge is generated during the process. Currently, most of the polymeric flocculants used in industry are synthetic, based on acrylamide copolymers.[149] Anionic polyacrylamide flocculants are mainly

copolymers of acrylamide and sodium acrylate, while the cationic type are copolymers of acrylamide with amine- or quaternized-amine monomers. Other kinds of cationic polymers synthesized from amines (dimethylamine) and epichlorohydrin or ethylenedichloride have been commercialized as well.

Although the use of synthetic polymers for flocculation applications is preferred over natural polymers for economic reasons, environmental concerns about degradability is slowly shifting the industry towards the use of the latter. Today, the use of natural polymers is limited to derivatized cationic starch (with diethylaminoethyl chloride or 2,3-epoxypropyltrimethylammonium chloride), nonionic starch and guar gum.[112,149] However, chitosan and some of its derivatives have shown very effective activities as flocculating agents in several studies (reviewed in chapter one), which makes them very likely candidates for industrial applications in the near future.

4.3 Flocculation activity.

The fact that kaolin clays provide the electrostatic requirements needed for flocculation studies, combined with their availability and low cost, makes them one of the materials of choice usually employed for

flocculation studies. These minerals are hydrated aluminum silicate clays ($Al_2O_3.xSiO_2.yH_2O$) such as kaolinite, dickite, nacrite and halloysite-endellite.[154,155] The sheet like particles contain anionic charges on the faces and cationic charges on the edges. When clay is dispersed in water, this electrostatic interaction between the opposite charges leads to slow aggregation of the particles,[156] which eventually settle down very slowly.

The criteria of successful flocculation varies with the application. The clarity of supernatants is the most important feature sought in the water treatment industry, while the speed of settling of the solids to be removed is the primary requirement in the mining industry. The main problem to solve in sewage applications, is the filterability and dewatering of the flocculated solids.[150] Therefore, we decided to determine flocculation activity in three different ways, by measuring settling rate, sediment volume, and supernatant clarity.[150,157-159] The equations used for calculating the parameters measured are:

settling rate (mL/sec.) = based upon the time required to settle 10 mL of the suspension volume.

normalized settling rate = settling rate of sample/settling rate standard

change in sediment volume = treated sample vol. (mL) - std. vol. (mL)

change in supernatant clarity = % T treated sample - % T std.

It is observed in Plot 4.1 that chitosan does increase the settling speed of kaolin clay when added to the suspension (a value of 1 would indicate same settling speed). Also, while most of the chitosan samples show an increase in the settling rate as the pH increases, chitosan obtained from Dr. Laine's laboratory did not show dependence on the pH. Although this sample exhibits a comparable percentage of deacetylation, the method of preparation produced a very heterogeneous distribution of the free amino groups along the chain. When this sample was dissolved in acetic acid, an insoluble residue remained.

The effectiveness of chitosan as a flocculating agent is corroborated in Plot 4.2, where significant decreases in the sediment volume were obtained when chitosan was added to the suspension. The data obtained does not indicate any dependence of the sediment volume on the pH.

In general, chitosan treatment raised the clarity of the supernatant layer to greater than 95% transmission. Consistent with the results

obtained previously, the polysaccharide shows that it can effectively remove clay particles from the suspension.

Table 4.1 Settling rate of chitosan.

chitin source	% deacetyl-ation	pH	std. time to settle 10 mL (sec.)	treated sample settling time (sec.)	norma-lized settling rate
Sigma	79	2.52	605	318	1.824
Sigma	79	5.02	890	405	2.273
Vanson	64	2.56	696	400	1.786
Vanson	64	4.99	769	318	2.385
Flonac	69	2.47	634	365	1.688
Flonac	69	5.08	790	280	2.769
Laine*	72	2.47	663	284	2.333
Laine*	72	4.98	818	355	2.333

* Measured on soluble component.

Table 4.2 Sediment volume of chitosan.

chitin source	% deacetyl-ation	pH	std. vol. (mL)**	treated sample vol. (mL)**	ΔV
Sigma	79	2.52	74	59	15
Sigma	79	5.02	76	59.5	16.5
Vanson	64	2.56	79	59.5	19.5
Vanson	64	4.99	74	56	18
Flonac	69	2.47	78.5	60	18.5
Flonac	69	5.08	77	60	17
Laine*	72	2.47	78	56.5	21.5
Laine*	72	4.98	77	58	19

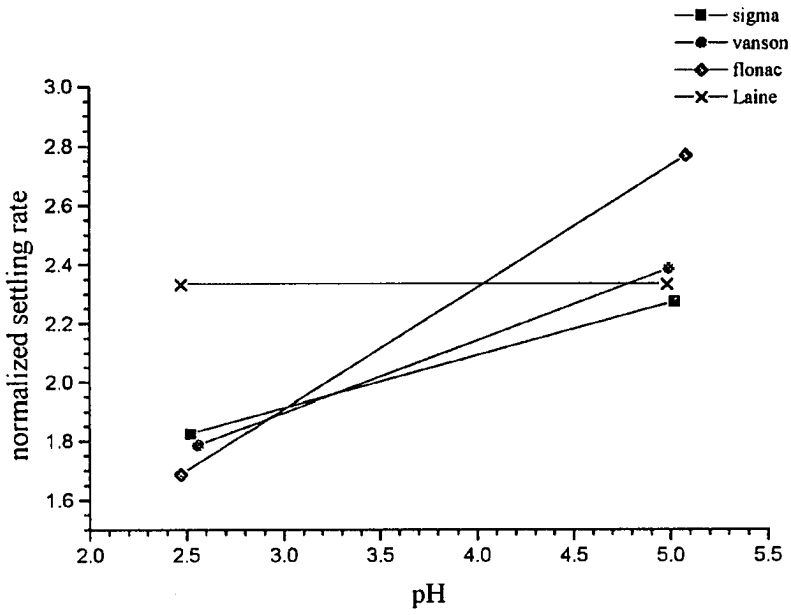
* Measured on soluble component.

** Volume of sediment in standard or treated sample after standing for 30 min.

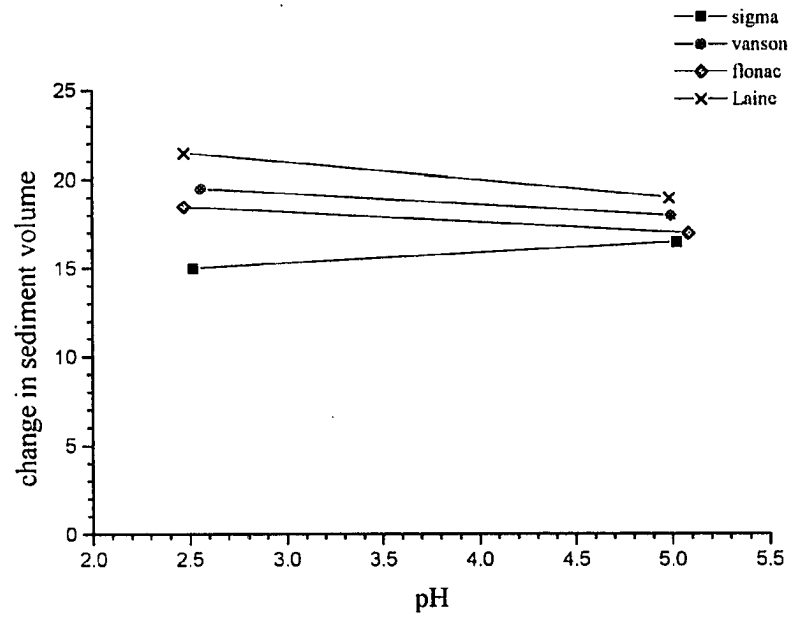
Table 4.3 Supernatant clarity of chitosan.

chitin source	% deacetyl-ation	pH	std. (% T)	treated sample (% T)	Δ % T
Sigma	79	2.52	93.76	94.19	0.43
Sigma	79	5.02	85.70	97.05	11.35
Vanson	64	2.56	94.41	97.05	2.64
Vanson	64	4.99	63.83	96.61	32.78
Flonac	69	2.47	95.06	97.05	1.99
Flonac	69	5.08	85.51	97.95	12.44
Laine*	72	2.47	92.68	96.16	3.48
Laine*	72	4.98	85.90	95.94	11.69

* Measured on soluble component.



Plot 4.1 Normalized settling rate of chitosan samples.



Plot 4.2 Change in sediment volume of chitosan samples.

Both derivatives studied show in Plot 4.3 a higher settling rate than chitosan at pH = 2.5, thus indicating their flocculation activity. However, opposite to the trend obtained for chitosan, the settling rate decreases as the pH increases to the extent that at pH = 5, both derivatives show less activity than any chitosan sample.

The change in sediment volume (Plot 4.4) for the derivatives obtained shows the same general behavior as chitosan, activity at low pH which decreases at higher pH values. However, hydroxypropyl chitosan is still active at basic pH in spite of the fact that this polymer is neutral. R-chitosan-quat 188, a cationic polymer, loses its activity at high pH although one would expect the retention of the positive charge should preserve its flocculation activity.

Similar to chitosan, both derivatives increase the supernatant clarity in the clay suspension at pH below 5.5. As the pH increases only partial flocculation is achieved and the residual, finely divided suspension reduces the clarity significantly.

In conclusion, depending on the property of interest, settling rate, sediment volume or supernatant clarity, and the pH of the suspension in question, chitosan, R-chitosan-quat 188 or hydroxypropyl chitosan, can

Table 4.4 Settling rate of chitosan-quat 188.

pH	std. time to settle 10 mL (sec.)	treated sample settling time (sec.)	normalized settling rate
2.50	559	230	2.389
5.07	1080	720	1.555
6.33	810	780	1.083
7.53	780	1455	0.538
9.97	----	----	----

Table 4.5 Settling rate of hydroxypropyl chitosan.

pH	std. time to settle 10 mL (sec.)	treated sample settling time (sec.)	normalized settling rate
2.56	585	302	1.941
5.08	795	564	1.385
6.48	730	715	1.00
7.56	----	1280	----
9.90	----	----	----

Table 4.6 Sediment volume of chitosan-quat 188.

pH	std. vol. (mL)**	treated sample vol. (mL)**	ΔV
2.50	80	68	12
5.07	86	80	6
6.33	76	72.5	3.5
7.53	73.5	87.5	-14
9.97	----	----	----

Table 4.7 Sediment volume of hydroxypropyl chitosan.

pH	std. vol. (mL)**	treated sample vol. (mL)**	ΔV
2.56	77	69	8
5.08	79	69	10
6.48	73.5	68	5.5
7.56	99.5	86.5	13
9.90	99.5	92	7.5

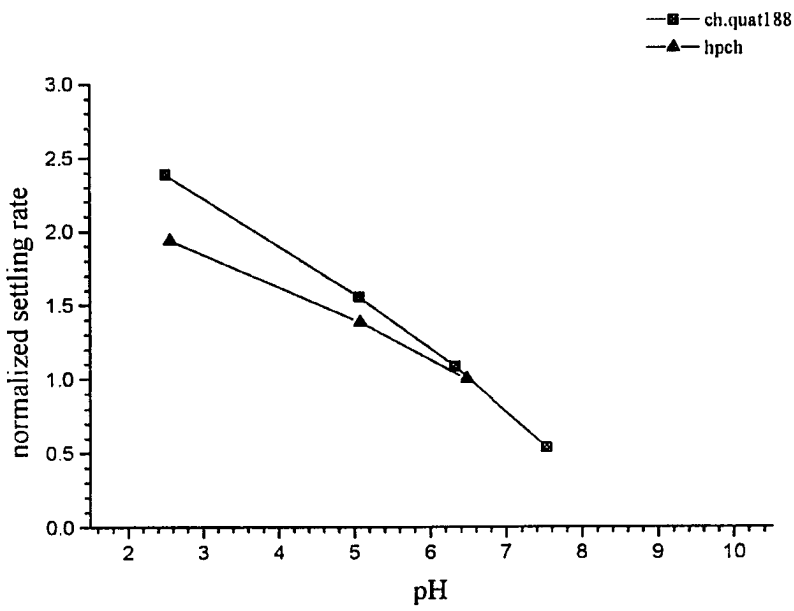
** Volume of sediment in standard or treated sample after standing for 30 min.

Table 4.8 Supernatant clarity of chitosan-quat 188.

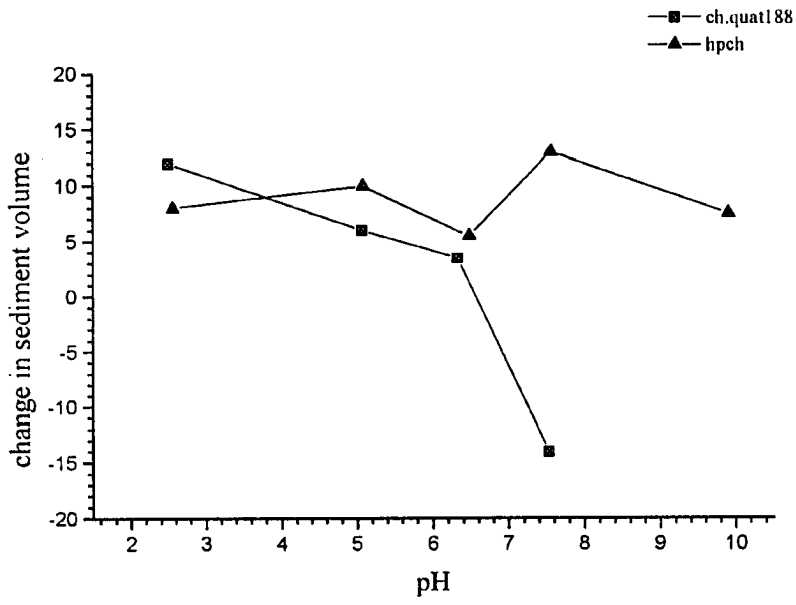
pH	standard (% T.)	treated sample (% T.)	Δ % T
2.50	95.28	94.19	-1.09
5.07	87.90	94.41	6.51
6.33	19.86	51.76	31.9
7.53	0.00	0.19	0.19
9.97	0.00	0.00	0.00

Table 4.9 Supernatant clarity of hydroxypropyl chitosan.

pH	standard (% T.)	treated sample (% T.)	Δ % T
2.56	94.19	91.41	-2.78
5.08	85.31	92.68	7.37
6.48	0.06	0.43	0.37
7.56	0.00	0.03	0.03
9.90	0.00	0.05	0.05



Plot 4.3 Normalized settling rate of R-ch-quat 188 and Hpch.



Plot 4.4 Change in sediment volume of R-ch-quat 188 and Hpch.

be used effectively as flocculation agents. It is noteworthy the fact that at basic pH, where chitosan does not dissolve and the R-chitosan-quat 188 activity is low, hydroxypropyl chitosan shows flocculation activity due to its neutral character. However, the modest activity observed suggests that this derivative would not be a practical flocculant. Potential applications in more expensive products, such as cosmetics, are being explored.

CHAPTER FIVE

EXPERIMENTAL

5.1 Instrumentation.

Infrared spectra were recorded with a Perkin Elmer FT-IR Spectrometer 1760X at a 4 cm^{-1} resolution and 10 scans. Nuclear Magnetic Resonance spectra were obtained using a Bruker AM 400 instrument for all the samples, except for the deaminated products, where a Bruker ARX 300 was used. Freeze drying purification of the samples was performed in a VirTis Freezemobile 12XL. Intrinsic viscosities were determined with a Ubbelohde viscometer # 50 Z107, the solvent flow time was 246.46 seconds. Cone and plate viscosities were obtained in Brookfield Digital Viscometer model LVTDCP. Thermal Analysis was performed in a Seiko TG/DTA 220. Differential Scanning Calorimetry was obtained using a Seiko DSC 220C.

5.2 Reagents and solvents.

Membrane tubing Spectra/Por from Spectrum Medical Industries, with a molecular weight cut-off 6-8,000 was used for dialysis of the derivatives. Chitosan was purchased from Sigma. Deuterated acetic acid, deuterium oxide, 3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt

(DSS), ethylene oxide, propylene oxide, 1,2-epoxybutane, styrene oxide, acrylonitrile, lithium aluminum hydride and hydrogen peroxide were purchased from Aldrich. Acetic acid, methanol, ammonium hydroxide, sodium acetate, sodium hydroxide, ethyl ether, tetrahydrofuran, hydrochloric acid, sodium bicarbonate and sodium nitrite were purchased from EM Science. N-(3-Chloro-2-hydroxypropyl) trimethylammonium chloride (Quat 188) was provided by The Dow Chemical Co. Thiele kaolin was provided by the Thiele Kaolin Co. All the reagents and solvents were utilized without purification, except tetrahydrofuran, which was dried on potassium hydroxide overnight and then distilled.

5.3 Determination of the degree of deacetylation.

5.3.1 IR method.

Chitosan films were cast from 0.5-1% wt./vol. solutions of chitosan in 1% vol./vol. acetic acid/water solutions. The film was washed thoroughly with 50% methanol/50% ammonium hydroxide (5% ammonium hydroxide/water), water, and methanol. Then, it was dried overnight in a vacuum dessicator. IR spectra were obtained by placing the free standing films directly in the sample beam.

5.3.2 NMR method.

Chitosan (10 mg/mL) was dissolved in 1% deuterated acetic acid/99% deuterium oxide, using DSS as the reference for the assignment of chemical shifts. The NMR spectrum was obtained from this solution.

5.4 Regeneration of chitosan.

Chitosan was regenerated by dissolving 1 gram (0.006 mol eq.) in 100 mL of 15% Acetic acid for 30 min., then it was reprecipitated with 200 mL of 15% $NaHCO_3$ and 200 mL of methanol. The regenerated chitosan was recovered by filtration.

5.5 Synthesis of oxirane-chitosan derivatives.

5.5.1 Synthesis of hydroxyethyl chitosan.

Chitosan (1g, 0.006 mol eq.) was swollen at r.t. for 24 hrs. with 15% NaOH (5 mL, 0.02 moles) and 50 mL of Isopropyl alcohol (IPA). Then, ethylene oxide (40 mL, 0.8 moles) was added to the reaction which was refluxed. A dry ice-acetone condenser was employed to recycle unreacted ethylene oxide for 6 hrs., and then a water cooled condenser which allowed the excess ethylene oxide to evaporate, was installed. The reaction was kept 48 hrs. at r.t. and then neutralized with

15% Acetic acid; placed into 100 mL of water and stirred for 30 min. A clear solution was obtained; low molecular weight impurities were removed by dialysis and freeze-drying; 2.97 g of hydroxyethyl chitosan was obtained.

5.5.2 Synthesis of hydroxyethyl chitosan using regenerated chitosan.

Fresh regenerated chitosan (1g, 0.006 mol eq.) was added to the reaction flask followed by addition of 40 mL of water, 15% NaOH (5 mL, 0.02 moles) and ethylene oxide (40 mL, 0.8 moles). The reaction was kept at r.t. for 4 hrs. using a dry ice-acetone condenser. At this moment, 60 mL of water were added to the reaction mixture, and the condenser was changed to a water cooled condenser. The reaction was kept at r.t. for a total of 24 hrs. followed by neutralization with 15% Acetic acid, dialysis, and freeze-drying; 1.32 g of R-hydroxyethyl chitosan was recovered.

5.5.3 Synthesis of hydroxypropyl chitosan.

Chitosan (1g, 0.006 mol eq.) was added to the reaction flask followed by addition of 15% NaOH (5 mL, 0.02 moles), propylene oxide (40 mL, 0.57 moles) and 50 mL of IPA. The reaction was kept at r.t. for 24 hrs.followed by 48 hrs. at 50°C; then, it was cooled down to r.t. and

neutralized with 15% Acetic acid. The reaction mixture was placed into 100 mL of water and dissolved by stirring for 30 min. Purification by dialysis followed by freeze-drying, yielded 1.84 g of hydroxypropyl chitosan.

5.5.4 Synthesis of hydroxypropyl chitosan using regenerated chitosan.

Fresh regenerated chitosan (1g, 0.006 mol eq.) was added to the reaction flask followed by addition of propylene oxide (40 mL, 0.57 moles) and 15% NaOH (5 mL, 0.02 moles) The reaction was kept at r.t. for 4 hrs. After addition of 100 mL of water, the temperature was raised to 45°C and held at that temperature for 20 hrs. (total reaction time 24 hrs.). The reaction was cooled down to r.t. and neutralized with 15% Acetic acid, dialyzed and freeze-dried; 1.60 g of R-hydroxypropyl chitosan was obtained.

5.5.5 Synthesis of hydroxybutyl chitosan.

Chitosan (1g, 0.006 mol eq.) was dissolved in 15% Acetic acid (100 mL) for 30 min.; then, 1,2-epoxybutane (40 mL, 0.46 moles) and 10 mL of methanol were added to the reaction flask. The total reaction time was 72 hrs. at 90°C; the reaction mixture was cooled down to r.t.

prior to neutralization with 15% NaOH, which was followed by dialysis and freeze-drying; 0.96 g of hydroxybutyl chitosan was recovered.

5.5.6 Synthesis of hydroxy(2-phenyl)ethyl chitosan.

Chitosan (1g, 0.006 mol eq.) was dissolved in 15% Acetic acid (100 mL) for 30 min.; then styrene oxide (40 mL, 0.35 moles) and 10 mL of methanol were added to the reaction flask. After 72 hrs. at 90°C the reaction mixture was cooled down to r.t and neutralized with NaOH 15%. The product was filtered and washed two times with 200 mL of water, followed by several washings with 200 mL of methanol until the alcohol from the washings was clear. The product was vacuum dried at r.t. overnight, obtaining 1.50 g of hydroxy(2-phenyl)ethyl chitosan.

5.5.7 Synthesis of regenerated chitosan-quat 188 using regenerated chitosan.

Fresh regenerated chitosan (1g, 0.006 mol eq.) was added to the reaction flask followed by addition of quat 188 (40 mL, 0.16 moles) and 15% NaOH (10 mL, 0.04 moles). The reaction was allowed to proceed at r.t. for 24 hrs. before addition of 100 mL of water and increasing the temperature to 50°C for 24 hrs. Then, the reaction was cooled down to

r.t., and neutralized with 15% Acetic acid followed by dialysis and freeze-drying; 1.27 g of R-chitosan-quat 188 was obtained.

5.6 Synthesis of acrylonitrile-chitosan derivatives.

5.6.1 Synthesis of cyanoethyl chitosan.

Chitosan (4 g, 0.023 mol eq.) was reacted with 15% NaOH (20 mL, 0.08 moles) and acrylonitrile (140 mL, 2.13 moles) for 24 hrs. at r.t. The reaction mixture was filtered and the flakes obtained were placed into 400 mL of water; filtered and washed again with 400 mL of water and 400 mL of methanol. After this process, the product was vacuum dried at r.t. overnight, obtaining 5.97 g of cyanoethyl chitosan.

5.6.2 Synthesis of cyanoethyl chitosan using regenerated chitosan.

Fresh regenerated chitosan (1g, 0.006 mol eq.) was added to the reaction flask followed by addition of acrylonitrile (35 mL, 0.53 moles) and 15% NaOH (5 mL, 0.02 moles). The reaction proceeded at r.t. for 4 hrs.; then the slurry was placed into 150 mL of water and stirred for 1 hr. After neutralization with 15% Acetic acid followed by dialysis and freeze-drying; 0.99 g of R-cyanoethyl chitosan was recovered.

5.6.3 Synthesis of aminopropyl chitosan.

Cyanoethyl chitosan (3 g) was suspended in 180 mL of ethyl ether and 150 mL of tetrahydrofuran followed by addition of lithium aluminum hydride (11.7 g, 0.31 moles) under nitrogen. The reaction was run for 72 hrs. at r.t.; then, 10 mL of water were added followed by filtration of the reaction mixture. The reaction mixture was transferred into 400 mL of 25% HCl, filtered again, and placed into 200 mL of 50% NH_4OH. Finally, the product was filtered and vacuum dried at r.t. overnight, obtaining 1.15 g of aminopropyl chitosan.

5.6.4 Synthesis of regenerated carboxyamidoethyl chitosan.

Regenerated cyanoethyl chitosan (1 g) was added to the reaction flask followed by addition of 30% H_2O_2 (300 mL, 2.94 moles) and NaOH (45 g, 1.13 moles) over a period of 1 hr. while the reaction flask was submerged in an ice-water bath. The reaction was kept in the ice bath for another 30 min.; then it was heated at 50°C for 3 hrs., cooled down to r.t. and neutralized with concentrated HCl. After dialysis and freeze-drying; 0.90 g of R-carboxyamidoethyl chitosan was obtained.

5.6.5 Selective hydrolysis of cyanoethyl chitosan (synthesis of regenerated carboxyethyl chitosan).

Regenerated cyanoethyl chitosan (1 g) was added to the reaction flask followed by addition of NaOH (45 g, 1.13 moles). The reaction was kept at 55°C for 5 hrs.; after this time, argon or air was blown into the system to sparge the ammonia being produced. Total reaction time was 24 hrs. at 55°C. After allowing the mixture to cool to r.t., and neutralizing with concentrated HCl, purification was performed by dialysis and freeze-drying; 0.48 g of R-carboxyethyl chitosan was isolated.

5.7 Characterization of oxirane and acrylonitrile derivatives of chitosan by FTIR.

Films of the derivatives obtained were cast from 0.5-1% wt./vol. solutions in either, 1% vol./vol. acetic acid/water solutions or water, depending on their solubilities. After the film was obtained, it was dried overnight at r.t. in a vacuum dessicator. The IR spectra were recorded by placing these films directly in the sample beam.

5.8 Characterization of oxirane and acrylonitrile derivatives of chitosan by NMR.

The solvents used for obtaining the NMR spectra depended on sample solubility. The sample (5-10 mg/mL) was dissolved in either, 1% deuterated acetic acid/99% deuterium oxide or deuterium oxide, using DSS as the reference for the assignment of chemical shifts.

5.9 Deamination of chitosan, regenerated cyanoethyl chitosan, and hydroxypropyl chitosan.

The corresponding sample (0.2 g) was dissolved in 15% Acetic acid (10 mL) followed by addition of 2 mL of 6M Hydrochloric acid. $NaNO_2$ (0.2 g, 2.9 mmoles)/10 mL of water was added to the solution, while this was kept in an ice-water bath. The reaction was left a minimum of 4 hrs. until nitrous acid was no longer detected; the reaction mixture was transferred to a Petri dish to evaporate the solvent. The physical appearance of the product was crystalline (no film was produced).

5.10 Viscosity measurements.

Chitosan and the derivatives obtained were dissolved in 0.2M Acetic acid/0.1M Sodium acetate. The concentrations used were: chitosan = 0.4976%, hydroxyethyl chitosan = 0.5084%, regenerated

hydroxyethyl chitosan = 0.5028%, hydroxypropyl chitosan = 0.5008%, regenerated hydroxypropyl chitosan = 0.4992%, hydroxybutyl chitosan = 0.5196%, regenerated chitosan-quat 188 = 0.5028%, regenerated cyanoethyl chitosan = 0.5024%, regenerated carboxyamidoethyl chitosan = 0.4972%. The determination of intrinsic viscosities was performed using an Ubbelohde viscometer at $25^0C \pm 0.02°C$, while the cone and plate viscosities were obtained utilizing a Brookfield viscometer at $25^0C \pm 0.02°C$.

5.11 Determination of TG and DSC.

Thermogravimetric analysis was performed with a Seiko TG/DTA 220. Chitosan and and all the derivatives studied were heated from r.t. to 450°C at 5°C/min. in air.

Differential scanning calorimetry was performed with a Seiko DSC 220C. All the samples were subjected to a two step process. The first step involved cooling from r.t. to -120°C (held for 0.5 min. at this temperature once it was reached) at a rate of 10°C/min. followed by heating from -120°C to 220°C at a rate of 10°C/min; both processes were performed in air.

5.12 Flocculation.

Thiele kaolin (40 g) and Aldrich kaolin (40 g) were suspended in tap water (1000 mL). The stock solution was allowed to stand overnight. The kaolin clay suspension was added to a 100 mL graduated cylinder and the settling rate, sediment volume and the supernatant clarity were measured. These parameters were measured after inverting the graduated cylinder 12 times to a 180° and monitoring the meniscus. The settling rate (mL/sec.) was determined by the amount of time it takes for the meniscus to reach 90 mL. The sediment volume (mL) was measured after allowing the meniscus to settle for 30 min. and then reading the volume of clay. The supernatant clarity was determined at the end of 30 min. by measuring absorption at 450 nm for the liquid above the sediment volume. To determine the effect the flocculating agent had on the kaolin clay suspension, the parameters were determined in all experimental runs prior to the addition of the flocculating agent.

Several samples were tested for their flocculation activity. Chitosan from Vanson, Flonac, Sigma and Dr. Laine's laboratory were dissolved in 15% Acetic acid to form 0.5% wt./vol. solutions. Hydroxypropyl chitosan and regenerated chitosan-quat 188 samples

were dissolved in distilled water to form 0.5% wt./vol. solutions. To the graduated cylinders containing the kaolin clay, 2 mL of these solutions were added, and the settling rate, sediment volume and the supernatant clarity were measured.

CHAPTER SIX

CONCLUSIONS

Chitin with a degree of deacetylation below 25% is a very intractable material, which due to its strong hydrogen bonding is unreactive and hard to solubilize. In order to produce a reactive chitosan, which allows derivatization, extensive deacetylation (about 75 %) of chitin is required. Furthermore, even though we could obtain oxirane and acrylonitrile derivatives of chitosan while utilizing flakes, regeneration of chitosan improves its reactivity. Reactions take place in less time, in aqueous solvents and the products exhibit improved solubilities.

The main objective, which was to design synthetic methodologies to obtain water soluble chitosan derivatives under mild conditions was achieved. Modifications of chitosan with epoxides catalyzed by base yielded products with better solubilities in water, while modifications under acidic conditions yielded products with solubilities under acidic media only. Derivatization of chitosan with acrylonitrile gave a product which swells in water. Attempts to react the cyanofunction further were accompanied by side reactions which degraded the polysaccharide backbone or removed the functional group. It was observed in the NMR

spectra, and corroborated by deamination, that oxirane-chitosan derivatives obtained under basic conditions are substituted at the primary amine in position 2. The derivatives obtained under acidic conditions and the acrylonitrile derivative contain the substituent at the primary alcohol in position 6.

Viscosity, and thermal analysis determinations demonstrated that even though the conditions utilized during the synthetic methods are very mild, some deacetylation and degradation occurs during the process.

Chitosan proved to be an effective flocculation agent for kaolin, but it is limited to acidic pH values due to its solubility. However, the water soluble 2-hydroxypropyl trimethylammonium chitosan chloride and hydroxypropyl chitosan, show flocculation activity at acidic pH values as well as under basic conditions.

BIBLIOGRAPHY

1.- R. A. A. Muzzarelli, Chitin, Pergamon, New York, 1977.

2.- R. A. A. Muzzarelli and E. R. Pariser, eds., Proceedings of the First International Conference on Chitin/Chitosan, M.I.T. Sea Grant Report MITSG 78-7, Cambridge, 1978.

3.- S. Hirano and S. Tokura, eds., Chitin and Chitosan and the Related Enzymes, The Japanese Society of Chitin and Chitosan, Tottori, 1982.

4.- R. A. A. Muzzarelli, C. Jeuniaux and G. W. Gooday, eds., Chitin in Nature and Technology, Plenum, New York, 1986.

5.- G. Skjak-Braek, T. Anthonsen and P. Sandford, eds., Chitin and Chitosan, Sources, Chemistry, Biochemistry, Physical Properties and Applications, Elsevier Applied Science, London, 1989.

6.- C. J. Brine, P. A. Sandford and J. P. Zikakis, eds., Advances in Chitin and Chitosan, Elsevier Applied Science, London, 1992.

7.- G. O. Aspinall, "General Introduction", in G. O. Aspinall, ed.,The Polysaccharides, Academic, New York, 1982, 1, 1-18.

8.- T. W. G. Solomons, Organic Chemistry, Fifth Edition, John Wiley & Sons,1992, 1030-1037.

9.- M. Yalpani, Polysaccharides, Syntheses, Modifications and Structure/Property Relations, Elsevier Science, New York, 1988, 8-49.

10.- J. F. Kennedy and C. A. White, Bioactive Carbohydrates: In Chemistry, Biochemistry and Biology, Ellis Horwood, West Sussex, 1983, 142-181.

11.- S. Gedon and R. Fengl, "Cellulose Esters", in J. I. Kroschwitz and M. Howe-Grant, eds., Kirk-Othmer Encycl. Chem. Technol., Fourth Edition, John Wiley & Sons, New York, 1993, 5, 496-540.

12.- K. Ward, Jr. and P. A. Seib, "Cellulose, Lichenan, and Chitin", in W. Pigman, D. Horton and A. Herp, ed., The Carbohydrates, Chemistry and Biochemistry, Second Edition, Academic, New York, 1970, 413-445.

13.- L. Stryer, Biochemistry, Third Edition, W. H. Freeman and Company, New York,1988, 449-468.

14.- Q. Li, E. T. Dunn, E. W. Grandmaison and M. F. A. Goosen, *J. Bioact. Comp. Polymers*, **1992**, 7, 370-397.

15.- C. Jeuniaux, M. C. Voss-Foucart, M. Poulicek and J. C. Bussers, "Sources of Chitin, Estimated from New Data on Chitin Biomass and Production", in Ref. 5, 3-11.

16.- D. Knorr, *Food Technology*, **1984**, 85-97.

17.- G. Goffinet and P. Compere, "Pore Canals and Organization of Chitinoproteins in the Cuticle of the Crab Carcinus maenas", in Ref. 4, 37-43.

18.- M. Poulicek, M. F. Voss-Foucart and C. Jeuniaux, "Chitinoproteic Complexes and Mineralization in Mollusk Skeletal Structures", in Ref. 4, 7-12.

19.- S. Kushino and Y. Orihara, JP 63,63,388, 1988; *Chem. Abst.* **1989**, 110, 6389.

20.- K. Shimahara, K. Ohkouchi and M. Ikeda, "A New Isolation Method of Crustacean Chitin using a Proteolytic Bacterium, Pseudomonas maltophilia", in Ref. 3, 10-14.

21.- M. M. Brzeski, "Concept of Chitin/Chitosan Isolation from Antartic Krill/Euphausia superba dana/Shells on a Technical Scale", in Ref. 3, 15-20.

22.- S. Mima, M. Miya, R. Iwamoto and S. Yoshikawa, "Highly Deacetylated Chitin and its Properties", in Ref. 3, 21-25; *J. Appl. Polym. Sci.*, **1983**, 28, 1909-1917.

23.- Q. P. Peniston and E. L. Johnson, U. S. Patent 4,066,735, 1978; *Chem. Abst.* **1978**, 88, 102017.

24.- Q. P. Peniston and E. L. Johnson, U. S. Patent 3,862,122, 1975; *Chem. Abst.* **1975**, 83, 12707.

25.- H. K. No, S. P. Meyers and K. S. Lee, *J. Agric. Food Chem.*, **1989**, 37, 575-579.

26.- K. Kurita, K. Tomita, T. Tada, S. Ishii, S. I. Nishimura and K. Shimoda, *J. Polym. Sci. Part A: Polym. Chem.*, **1993**, 31, 485-491.

27.- A. Domard and M. Rinaudo, *Int. J. Biol. Macromol.*, **1983**, 5, 49-52.

28.- A. C. M. Wu and W. A. Bough, "A Study of Variables in the Chitosan Manufacturing Process in Relation to Molecular-Weight Distribution, Chemical Characteristics and Waste-Treatment Effectiveness", in Ref. 2, 88-102; W. A. Bough, W. L. Salter, A. C. M. Wu and B. E. Perkins, *Biotech. Bioeng.*, **1978**, 20, 1931-1943.

29.- M. L. Bade and K. Hickey, "Classification of Enzymes Hydrolyzing Chitin", in Ref. 5, 179-183.

30.- R. A. A. Muzzarelli, "Chitinases and Related Enzymes", in Ref. 1, 155-181.

31.- R. A. Smucker, "Interpretative Model for Enzymatic Chitin Hydrolysis", in Ref. 4, 254-258.

32.- A. Ohtakara and M. Mitsutomi, "Mode of Hydrolysis of Chitooligosaccharides with Chitinase and Chitobiase produced by Pycnoporus cinnabarinus", in Ref. 3, 117-121.

33.- D. Fenton, B. Davis, C. Rotgers and D. E. Eveleigh, "Enzymatic Hydrolysis of Chitosan", in Ref. 2, 525-541.

34.- K. M. Varum, G. Rosenlund and O. Smidsrod, "Enzymatic Degradation of Chitosan in Atlantic salmon Salmo salar", in Ref. 5, 299-308.

35.- E. Murakami, K. Hasegawa, J. Tamura and K. Kadowaki, "The Efficient Production of High DP Chitooligosaccharides by Enzymatic Hydrolysis of Chitosan", in Ref. 6, 259-267.

36.- Y. Uchida, K. Tateishi, O. Shida and K. Kadowaki, "Purification and Enzymatic Properties of Chitosanases from Bacillus licheniformis", in Ref. 6, 282-291.

37.- R. Tanaka, E. Muraki, S. Fujishima and F. Yaku, "Properties of Chitosanase from Cellulase Preparations", in Ref. 6, 276-281.

38.- D. Pantaleone, M. Yalpani and M. Scollar, "Susceptibility of Chitosan to Enzymatic Hydrolysis", in Ref. 6, 292-303.

39.- R. A. A. Muzzarelli, *Carbohydr. Polym.*, **1983**, 3, 53-75.

40.- H. Yabe, Y. Kawamura and I. Kurahashi, JP 63,161,001, 1988; *Chem. Abst.* **1989**, 110, 141288.

41.- G. Lang, G. Maresch and H. R. Lenz, Ger. Offen. DE 3,723,811, 1989; *Chem. Abst.* **1989**, 110, 236967.

42.- S. Matsumura, T. Karigome and K. Nomoto, JP 01,14,203, 1989; *Chem. Abst.* **1989**, 110, 215083.

43.- E. Onsoyen, *Parfums, Cosmet., Aromes*, **1991**, 97, 85-86.

44.- E. Onsoyen and O. Skaugrud, *Seifen, Oele, Fette, Wachse*, **1991**, 117, 633-637.

45.- G. Lang and H. Wendel, DE 3,644,097, 1988; *Chem. Abst.* **1989**, 110, 218811.

46.- S. Hirano, K. Hirochi, K. Hayashi, T. Mikami and H. Tachibana, "Cosmetic and pharmaceutical uses of chitin and chitosan", in C. G. Gebelein, T. C. Cheng and V. C. Yang, eds., *Cosmet. Pharm. Appl. Polym.*, Plenum, New York, **1991**, 95-104.

47.- G. Lang and H. Wendel, EP 290,741, 1988; *Chem. Abst.* **1989**, 110, 141254.

48.- M. Mizushima, JP 63,165,307, 1988; *Chem. Abst.* **1989**, 110, 198910.

49.- S. Iwao, JP 01,09,911, 1989; *Chem. Abst.* **1989**, 110, 218803.

50.- P. Gross, E. Konrad and H. Mager, "Investigations on Chitosan as a Natural Film Forming Ingredient in Hair Cosmetic Products under the Consideration of Ecological Aspects", in Ref. 3, 205-209.

51.- R. A. A. Muzzarelli, "Amphoteric Derivatives of Chitosan and their Biological Significance", in Ref. 5, 87-99.

52.- K. Shinagawa, G. Takemura and A. Kobayashi, JP 54,152,685, 1979; *Chem. Abst.* **1980**, 93, 53232.

53.- W. A. Bough and D. R. Landes, "Treatment of Food-Processing Wastes with Chitosan and Nutritional Evaluation of Coagulated By-Products", in Ref. 2, 218-230.

54.- T. Asano, N. Havakawa and T. Suzuki, "Chitosan Applications in Wastewater Sludge Treatment", in Ref. 2, , 231-252.

55.- K. L. Simpson, "The Recovery of Protein and Pigments from Shrimp and Crab Meals and their Use in Salmonid Pigmentation", in Ref. 2, 253-262.

56.- C. Senstad and K. A. Almas, "Use of Chitosan in the Recovery of Protein from Shrimp Processing Wastewater", in Ref. 4, 568-570.

57.- W. A. Bough, *J. Food Sci.*, **1975**, 40, 297.

58.- H. K. No and S. P. Meyers, *J. Agric. Food Chem.*, **1989**, 37, 580-583.

59.- H. K. No and S. P. Meyers, *J. Food Sci.*, **1989**, 54, 60-62, 70.

60.- R. A. A. Muzzarelli, "New Derivatives of Chitin and Chitosan: Properties and Applications", in V. Crescenzi, I. C. M. Dea and S. S. Stivala, eds., New Developments in Industrial Polysaccharides, Gordon and Breach Science, New York, 1985, 207-231.

61.- N. Nishi, S. Nishimura, Y. Maekita, A. Ebina, A. Tsutsumi and S. Tokura, "Preparation and Characterization of Phosphorylated Chitin and Chitosan", in Ref. 4, 297-299.

62.- R. A. A. Muzzarelli, "The Chelating Ability of Amino Acid Glucans and Sugar Acid Chitosans", in Ref. 4, 321-330.

63.- G. Vanlerberghe and H. Sebag, U. S. Patent 4,031,025, 1977; *Chem. Abst.* **1977**, 87, 58410.

64.- R. A. A. Muzzarelli, Natural Chelating Polymers; Alginic acid, Chitin, and Chitosan, Pergamon, New York, 1973, 83-95.

65.- R. A. A. Muzzarelli, F. Tanfani, G. Scarpini and E. Tucci, *J. Appl. Biochem.*, **1980**, 2, 54-59.

66.- T. Sakaguchi and A. Nakajima, "Recovery of Uranium by Chitin Phosphate and Chitosan Phosphate", in Ref. 3, 177-182.

67.- K. Ramachandran Nair and P. Madhavan, "Metal Binding Property of Chitosan from Different Sources", in Ref. 3,187-190.

68.- M. S. Masri and V. G. Randall, "Chitosan and Chitosan Derivatives for Removal of Toxic Metallic Ions from Manufacturing-Plant Waste Streams", in Ref. 2, 277-287.

69.- R. A. A. Muzzarelli and A. Zattoni, *Int. J. Biol. Macromol.*, **1986**, 8, 137-141.

70.- C. Peniche-Covas, L. W. Alvarez, W. Arguelles-Monal, *J. Appl. Polym. Sci.*, **1992**, 46, 1147-1150.

71.- K. Kurita, S. Chikaoka and Y. Koyama, *Chem. Lett.*, **1988**, 9-12.

72.- P. A. Sandford and A. Steinnes, "Biomedical Applications of High Purity Chitosan", in S. W. Shalaby, C. L. McCormick and G. B. Butler, eds., Water-Soluble Polymers, Synthesis, Solution Properties and Applications, American Chemical Society, Washington, D. C., 1991, 430-445.

73.- P. A. Sandford, "Chitosan: Commercial Uses and Potential Applications", in Ref. 5, 51-69.

74.- J. G. Domszy and G. A. F. Roberts, *Makromol. Chem.*, **1985**, 186, 1671-1677.

75.- K. Kurita, S. Nishimura, Y. Koyama, S. Inoue and O. Kohgo, *Polym. Prepr.*, **1990**, 31 (1), 624-625.

76.- E. R. Hayes and D. H. Davies, "Characterization of Chitosan. II: The Determination of the Degree of Acetylation of Chitosan and Chitin", in Ref. 2, 406-420.

77.- G. G. Maghami and G. A. F. Roberts, *Makromol. Chem.*, **1988**, 189, 2239-2243.

78.- R. A. A. Muzzarelli and R. Rocchetti, "The Determination of the Degree of Acetylation of Chitosans by Spectrophotometry", in Ref. 4, 385-388

79.- E. Curotto and F. Aros, *Anal. Biochem.*, **1993**, 211, 240-241.

80.- M. Miya, R. Iwamoto, S. Yoshikawa and S. Mima, *Int. J. Biol. Macromol.*, **1980**, 2, 323-324.

81.- T. D. Rathke and S. M. Hudson, *J. Polym. Sci. Part A: Polym. Chem.*, **1993**, 31, 749-753.

82.- G. K. Moore and G. A. F. Roberts, *Int. J. Biol. Macromol.*, **1980**, 2, 115-116.

83.- A. Baxter, M. Dillon, K. D. A. Taylor and G. A. F. Roberts, *Int. J. Biol. Macromol.*, **1992**, 14, 166-169.

84.- A. Domard, C. Gey, M. Rinaudo and C. Terrasin, *Int. J. Biol. Macromol.*, **1987**, 9, 233-237.

85.- M. Rinaudo, P. L. Dung, C. Gey and M. Milas, *Int. J. Biol. Macromol.*, **1992**, 14, 122-128.

86.- M. Yalpani, Polysaccharides, Syntheses, Modifications and Structure/Property Relations, Elsevier Science, New York, 1988, 189-231.

87.- C. Meister and R. Doenges, DE 3,903,797, 1990; *Chem. Abst.* **1991**, 114, 45362.

88.- G. Lang, H. Wendel and E. Konrad, DE 3,501,891, 1986; *Chem. Abst.* **1986**, 105, 139429.

89.- G. Lang, H. Wendel and E. Konrad, DE 3,502,833, 1986; *Chem. Abst.* **1986**, 105, 139430.

90.- E. M. Partain III and G. L. Brode II, WO 8,707,618, 1987; *Chem. Abst.* **1989**, 110, 179564.

91.- G. L. Brode II, P. A. Band, E. D. Goddard, A. G. Barbone, A. Leshchiner, J. P. Pavlichko, E. M. Partain III and P. S. Leung, US Patent 4,767,463 1988; *Chem. Abst.* **1989**, 110, 121097.

92.- E. K. Just and T. G. Majewicz, "Cellulose ethers", in J. I. Kroschwitz, ed., Encycl. Polym. Sci. Eng., John Wiley & Sons, New York, 1985, 3, 226-269.

93.- R. A. A. Muzzarelli, "Chitin Chemistry", in Ref. 1, 87-154.

94.- G. Lang, E. Konrad and H. Wendel, "Chitosan Derivatives: Water Soluble Products by Reaction with Epoxides", in Ref. 4, 303-306.

95.- O. Somorin, N. Nishi and S. Tokura, "Preparation and Some Properties of Water Soluble Chitin Derivatives, in Ref. 3, 54-56.

96.- G. Maresch, T. Clausen and G. Lang, "Hydroxypropylation of Chitosan", in Ref. 5, 389-395.

97.- S. J. Kim, S. S. Kim and Y. M. Lee, *Macromol. Chem. Phys.*, **1994**, 195, 1687-1693.

98.- Hoechst A.-G., DE 4,310,088, 1994; *Chem. Abst.* **1995**, 122, 58652.

99.- T. Sannan, S. Tsuchida, H. Ise and S. Horiguchi, EP 585,885, 1994; *Chem. Abst.* **1994**, 120, 226527.

100.- G. Lang, G. Maresch and H. R. Lenz, DE 3,723,811, 1989; *Chem. Abst.* **1989**, 110, 236967.

101.- Y. Asao, JP 61,060,701, 1986; *Chem. Abst.* **1986**, 105, 116911.

102.- G. Lang, G. Maresch, H. Wendel, H. R. Lenz and E. Konrad, DE 3,602,402, 1987; *Chem. Abst.* **1988**, 108, 156266.

103.- H. Morita, A. Kasahara and Y. Ishizaki, JP 61,285,258, 1986; *Chem. Abst.* **1987**, 107, 60089.

104.- S. Iwase, JP 62,064,803, 1987; *Chem. Abst.* **1987**, 107, 25061.

105.- S. Tsuchida, H. Ise, T. Yamanami and S. Horiguchi, JP 06,065,304, 1994; *Chem. Abst.* **1994**, 121, 60101.

106.- T. Tian-Rui, K. Inouye, Y. Machida, T. Sannan and T. Nagai, *Yakuzaigaku*, **1988**, 48, 318-321; *Chem. Abst.* **1989**, 110, 237012.

107.- G. Lang, G. Maresch, H. Wendel, E. Konrad, H. R. Lenz and J. Titze, DE 3,541,305, 1987; *Chem. Abst.* **1987**, 107, 102453.

108.- T. Yamanami and S. Horiguchi, JP 03,234,702, 1991; *Chem. Abst.* **1992**, 116, 23284.

109.- Nippon Soda Co., JP 57,180,602, 1982; *Chem. Abst.* **1983**, 98, 181486.

110.- S. Ogino, N. Morya and N. Harada, JP 05,139,939, 1993; *Chem. Abst.* **1993**, 119, 146339.

111.- Y. Chikamatsu, Y. Ando, K. Hasebe, M. Inagaki, K. Katsura, I. Takano and H. Kizawa, JP 02,025,409, 1990; *Chem. Abst.* **1990**, 112, 204459.

112.- The Dow Chemical Co., Quat 188 Cationic Reagent, Technical Information, 1-21.

113.- L. A. Nud'ga, E. A. Plisko and S. N. Danilov, *Zhur. Obs. Khim.*, **1975**, 45, 1145-1149.

114.- S. Tokura, N. Nishi, S. Nishimura and Y. Ikeuchi, *Polym. J.*, **1983**, 15, 553-556.

115.- L. Zheng and J. Yang, *Shiyou Huagong*, **1991**, 20, 687-690; Chem. Abstr. **1991**, 116, 236595.

116.- W. H. Daly and A. Munir, *J. Polym. Sci. Polym. Chem. Ed.*, **1984**, 22, 975-984.

117.- J. March, Advanced Organic Chemistry, Third Edition, John Wiley & Sons, New York, 1985, 780-872.

118.- C. R. Noller, Org. Synth., Coll. Vol. II, 1943, 586-587.

119.- J. E. McIsaac Jr., R. E. Ball and E. J. Behrman, J. Org. Chem., 1971, 36, 3048-3050.

120.- J. S. Buck and W. S. Ide, Org. Synth., Coll. Vol. II, 1943, 44-46.

121.- S. Cacchi, D. Misiti and F. La Torre, *Synthesis*, **1980**, 243-244.

122.- N. Weiner, Org. Synth., Coll. Vol. II, 1943, 376-378.

123.- R. R. Read, Org. Synth., Coll. Vol. I, 1941, 321-322.

124.- C. Erbing, B. Lindberg and S. Svensson, *Acta Chem. Scand.*, **1973**, 27, 3699-3704.

125.- C. Plassard, C. Vignon, D. Mousain and L. Salsac, "Glucosamine Determination as a Means of Measuring Fungal Mass and Mycorrhizal Infection", in Ref. 4, 95-98.

126.- G. G. Allan and M. Peyron, "The Kinetics of The Depolymerization of Chitosan by Nitrous Acid", in Ref. 5, 443-466.

127.- E. R. Hayes, D. H. Davies and V. G. Munroe, "Organic Acid Solvent Systems for Chitosan", in Ref. 2, 103-106.

128.- M. Rinaudo and A. Domard, "Solution Properties of Chitosan", in Ref. 5, 71-86.

129.- R. A. A. Muzzarelli, "Chitin", in Aspinall G. O., ed., The Polysaccharides, Academic, 1985, 3, 417-453.

130.- A. Domard, *Int. J. Biol. Macromol.*, **1987**, 9, 98-104.

131.- P. J. Flory, Principles of Polymer Chemistry, Cornell University Press, Ithaca, 1953, 266-316.

132.- G. G. Maghami and G. A. F. Roberts, *Makromol. Chem.*, **1988**, 189, 195-200.

133.- W. Wang, S. Bo, S. Li and W. Qin, *Int. J. Biol. Macromol.*, **1991**, 13, 281-285.

134.- N. Errington, S. E. Harding, K. M. Varum and L. Illum, *Int. J. Biol. Macromol.*, **1993**, 15, 113-117.

135.- M. Rinaudo, M. Milas and P. L. Dung, *Int. J. Biol. Macromol.*, **1993**, 15, 281-285.

136.- R. G. Beri, J. Walker, E. T. Reese and J. E. Rollings, *Carbohydr. Res.*, **1993**, 238, 11-26.

137.- C. Wu, S. Zhou, W. Wang, *Biopolymers*, **1995**, 35, 385-392.

138.- F. Rodriguez, Principles of Polymer Systems, Third Edition, Hemisphere Publishing, New York, 1989, 177-227.

139.- C. K. Schoff, "Rheological Measurements", in J. I. Kroschwitz, ed., Concise Encycl. Polym. Sci. Eng., John Wiley & Sons, New York, 1990, 994-999.

140.- M. Yalpani, Polysaccharides, Syntheses, Modifications and Structure/Property Relations, Elsevier Science, New York, 1988, 83-141.

141.- J. N. BeMiller, "Gums, Industrial", in J. I. Kroschwitz, ed., Encycl. Polym. Sci. Eng., John Wiley & Sons, New York, 1985, 7, 589-613.

142.- J. H. Flynn, "Thermal Analysis", in J. I. Kroschwitz, ed., Concise Encycl. Polym. Sci. Eng., John Wiley & Sons, New York, 1990, 1182-1185.

143.- K. Ogura, T. Kanamoto, M. Itoh, H. Miyashiro and K. Tanaka, *Polym. Bull.*, **1980**, 2, 301-304.

144.- J. H. Kim and Y. M. Lee, *Polymer*, **1993**, 34 (9), 1952-1957.

145.- D. V. Luyen and V. Rossbach, *J. Appl. Polym. Sci.*, **1995**, 55, 679-685.

146.- G. Cardenas T., L. Bernal A. and L. H. Tagle D., *Termochim. Acta*, **1992**, 195, 33-38.

147.- C. Peniche-Covas, W. Arguelles-Monal and J. San Roman, *Polym. Degrad. Stab.*, **1993**, 39, 21-28.

148.- M. Pizzoli, G. Ceccorulli and M. Scandola, *Carbohydr. Res.*, **1991**, 222, 205-213.

149.- G. R. Rose and M. R. St. John, "Flocculation", in J. I. Kroschwitz, ed., Encycl. Polym. Sci. Eng., John Wiley & Sons, New York, 1985, 7, 211-233.

150.- P. F. Wilde and R. W. Dexter, *Br. Polym. J.*, **1972**, 4, 239-250.

151.- J. A. Kitchener, *Br. Polym. J.*, **1972**, 4, 217-229.

152.- A. D. Read, *Br. Polym. J.*, **1972**, 4, 253-264.

153.- A. Domard, M. Rinaudo and C. Terrassin, *J. Appl. Polym. Sci.*, **1989**, 38, 1799-1806.

154.- C. L. Rollinson, "Calcium compounds (survey)", in M. Grayson and D. Eckroth, ed., Kirk-Othmer Encycl. Chem. Technol., John Wiley & Sons, New York, Third Ed., 1978, 4, 421-426.

155.- W. D. Keller, "Clays (survey)", in M. Grayson and D. Eckroth, ed., Kirk-Othmer Encycl. Chem. Technol., John Wiley & Sons, New York, Third Ed., 1978, 6, 190-206.

156.- G. G. Spence, "Paper additives and resins", in J. I. Kroschwitz, ed., Encycl. Polym. Sci. Eng., John Wiley & Sons, New York, 1985, 10, 761-786.

157.- V. K. La Mer and R. H. Smellie, *J. of Coll. Sci.*, **1956**, 11, 704-709.

158.- R. H. Smellie and V. K. La Mer, *J. of Coll. Sci.*, **1956**, 11, 720-731.

159.- V. K. La Mer and R. H. Smellie, *J. of Coll. Sci.*, **1956**, 11, 710-719.

APPENDIX A

CALCULATION OF D. S. AND M. S.

Degree of substitution = $\frac{\text{average number of substituent groups}}{\text{anhydroglucose ring.}}$

Molar substitution = average size of chains attached to each anhydroglucose ring.

1.- Hydroxyethyl chitosan.

D. S. = 12.6 (H region 2.5-3)/3 (signal belongs to 3 H)

= 4.2/3.1 (H-1) = 1.35

M. S. = 144.2 (H region 3.3-4.2) + 8.4 (2 H region 2.5-3)

= 152.6/3.1 (H-1) = 49.23 (total H/H-1) - 4.70 (total H/H-1 in chitosan) = 44.53/4 (total H/ethylene unit) = 11.13

2.- Regenerated hydroxyethyl chitosan.

D. S. = 8.1 (H region 2.8-3.1)/2 (signal belongs to 2 H)

= 4.05/2.8 (H-2) = 1.45

M. S. = 54.3 (H region 3.4-4.3) + 8.1 (2H region 2.8-3.1)

= 62.4/2.8 (H-2) = 22.29 (total H/H-1) - 4.70 (total H/H-1 in chitosan) = 17.59/4 (total H/ethylene unit) = 4.4

3.- Hydroxypropyl chitosan.

D. S. = 10.6 (H region 2.5-3)/3 (signal belongs to 3 H)

= 3.53/3.5 (H-1) = 1

M. S. = 42 (H region 1-1.3)/3 (total H/methyl unit)

= 14/3.5 (H-1) = 4

4.- Regenerated Hydroxypropyl chitosan.

D. S. = 3.72 (H region 2.5-3)/3 (signal belongs to 3 H)

= 1.24/0.57 (H-1) = 2.18

M. S. = 9.59 (H region 0.9-1.3)/3 (total H/methyl unit)

= 3.2/0.57 (H-1) = 5.6

5.- Hydroxybutyl chitosan.

D. S. = 0.90 (H region 0.9-1)/3 (total H/methyl unit)

= 0.3/2.39 (H-2) = 0.13

6.- Hydroxy(2-phenyl)-ethyl chitosan.

D. S. = 0.979 (H region 2.8-3)/3 (signal belongs to 3 H in styrene

oxide) = 0.33/0.707 (H-2) = 0.47

7.- Regenerated chitosan-quat 188.

D. S. = 1.794 + 1.828 (average of H region 2.7-3)/2

= 1.811/1.676 (H-2) = 1.08

M. S. = 13.903 (H region 3.1-3.3)/9 (total H/3 methyl units)

= 1.54/1.676 (H-2) = 0.9

8.- Cyanoethyl chitosan.

D. S. = 13.22 (H region 2.7-3)/2 (signal belongs to 2 H)

= 6.61/3.38 (H-2) = 1.96

9.- Regenerated cyanoethyl chitosan.

D. S. = 5.81 (H region 2.7-3)/2 (signal belongs to 2 H)

= 2.91/2.94 (H-2) = 0.99

10.- Aminopropyl chitosan.

D. S. = 2.65 (H region 2.6-3)/2 (signal belongs to 2 H)

= 1.325/1.42 (H-2) = 0.93

11.- Regenerated carboxyamidoethyl chitosan.

D. S. = 11.434 (H region 2.4-2.7)/2 (signal belongs to 2 H)

= 5.72/4.015 (H-2) = 1.42

12.- Regenerated carboxyethyl chitosan.

D. S. = 0.0809 (H region 2.6-2.8)/2 (signal belongs to 2 H)

= 0.04/0.1937 (H-2) = 0.21

APPENDIX B

PRODUCTION COST ASSESSMENT OF CHITOSAN DERIVATIVES

An assessment of the cost associated with producing hydroxypropyl chitosan and chitosan-quat 188 appears below. The assessment is restricted to materials cost only, as no design criteria exist for production units.

Chemical	$/kg
Hydroxypropyl chitosan	65.42
Chitosan-Quat 188	76.15

These prices were calculated based on the following costs of chemicals and are corrected by the yield obtained for each derivative:

Chemical	$/kg
Chitosan	14.00
NaOH	2.26
Acetic acid	0.66
Isopropyl alcohol	0.52*
Propylene oxide	1.19
Quat 188	2.30

* in liters.

VITA

Javier Macossay Torres was born December 12, 1967, in Acapulco, Guerrero, Mexico. He received his Bachelor of Science degree in Chemistry from Universidad Autonoma de Nuevo Leon in San Nicolas de los Garza, Nuevo Leon, Mexico in August 1989. He continued his studies at Louisiana State University in Baton Rouge, Louisiana, under the supervision of Dr. William H. Daly from August 1990 to the present. He was awarded the Master of Science degree in Chemistry in August 1993, and is currently a candidate for the Doctor of Philosophy degree in the the area of Polymer Chemistry. He married Alma Delia Hernandez Ramos on May 17, 1990. The couple has been blessed with two children, Alma Maria Macossay Hernandez and Javier Jesus Macossay Hernandez.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Javier Macossay

Major Field: Chemistry

Title of Dissertation: Synthesis and Characterization of Water Soluble Chitosan Derivatives

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

August 29, 1995